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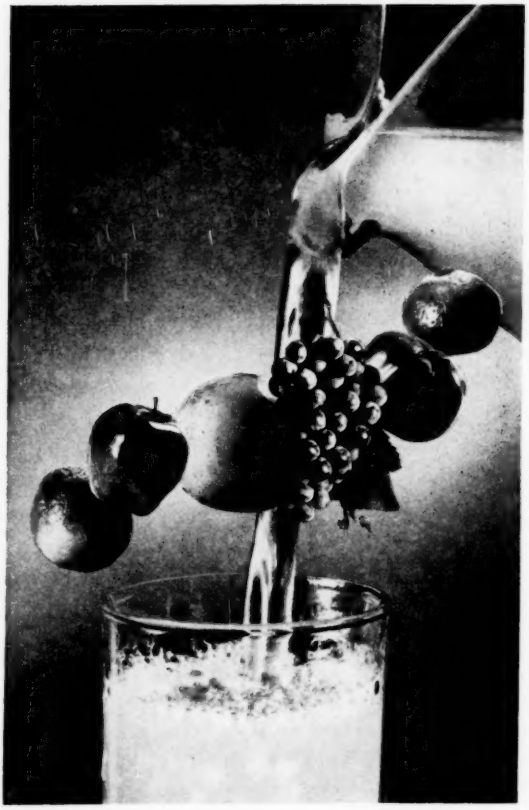

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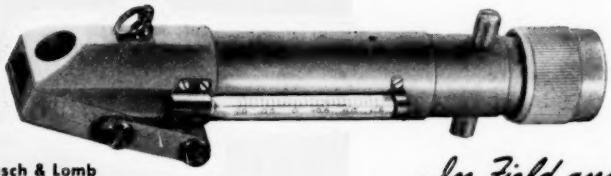

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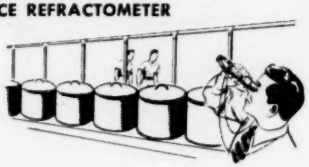

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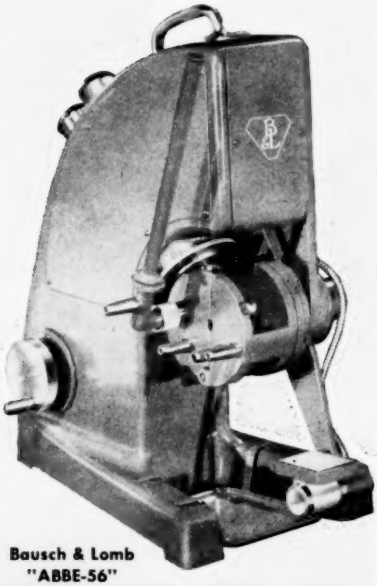

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THE EFFECT OF SELECTED PRETREATMENTS UPON THE CULINARY QUALITIES OF EGGS FROZEN AND STORED IN A HOME-TYPE FREEZER.

II. SPONGE CAKES[a]

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(Manuscript received April 3, 1951)

In the first phase of this study, the effect of various treatments of egg yolks or mixed whole eggs prior to freezing and storage upon their behavior in plain cakes and custards was considered *(2)*. The second part deals with the effect of these treatments upon the leavening property of the eggs as used in sponge cakes.

Godston *(1)* found that yolks treated with 3% glycerine or 7% sugar before being frozen produced sponge cakes of better volume and score than untreated yolks. Pearce and Reid *(6)* reported that volumes of sponge cakes made from whole eggs that had been frozen and stored for 3 months were greater than those of cakes made from eggs just frozen or frozen and stored for 6 months or longer.

Pearce and Lavers *(5)* found that freezing reduced the baking quality of yolk and whole egg, but the baking quality improved after storage for about 3 months, and then decreased; the addition of 2% sodium chloride or 8% sucrose to egg yolk before it was frozen improved the foaming volume of defrosted yolk combined with unfrozen white. Miller and Winter *(4)* concluded that pasteurization and freezing of liquid whole egg had no significant effect upon the quality of sponge cakes; their formula included a small amount of baking powder as secondary leavening agent.

Household formulas for sponge cakes usually call for the eggs to be separated into whites and yolks; however, with the more general availability of household electric mixing machines it is possible for the home baker to use the whole-egg meringue method of Pyke and Johnson *(7)*. In the study here reported cakes were made from both separated and unseparated eggs.

EXPERIMENTAL PROCEDURE

The experiment was designed to include 8 treatments (7 treatments of frozen egg samples and 1 fresh shell egg control), 3 replications, and 4 storage periods.

Eggs and their preparation. The eggs were from the same source as those used in the study on plain cakes and custards previously reported *(2)*. They were collected, frozen, and stored during the months of April and May.

As the eggs to be used in the separated-egg sponge cakes were broken out and scored, the yolks and whites were separated. The whites were blended with as little incorporation of air as feasible, strained through a household wire strainer, then packaged in 60-g. lots in waxed paper cartons with close-fitting lids. The yolks were broken, mixed, strained, and treated with 2 levels each of salt, sugar, and sirup as

[a]Journal Paper No. 531, Purdue University, Agricultural Experiment Station.
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described for those frozen for use in egg yolk custards *(2)*. The treated samples and the 1 untreated sample were weighed out into small cartons, each carton containing 36 g. of yolk plus the proportionate share of added ingredient.

The eggs for the whole-egg sponge cakes were prepared as the eggs used in the plain cakes and whole-egg custards *(2)*. They were packaged in cartons each of which contained 96 g. of mixed whole egg plus a proportionate share of 1 of the added ingredients.

The packaged eggs were placed in a home-type freezer, frozen and stored at a temperature of about 0°F.(—18°C.).

Samples for use in baking were withdrawn after frozen storage for periods of 1 week, and 2, 4, and 6 months. Each time cakes were baked from the defrosted eggs, 1 control cake was made from fresh shell eggs.

Separated-egg sponge cakes. The formula for the separated-egg sponge cake was as follows:

Egg white	60 g.
Egg yolk	36 g.
Sugar, fine granulated	70 g.
Flour, cake	35 g.
Salt, table-grade	0.5 g.
Cream of tartar	0.5 g.
Water	10-15 ml.

For the cakes made from frozen eggs, the whites and yolks were defrosted just before they were to be used.

The egg whites and the salt (salt was omitted from cakes made from salt-treated yolks) were put into the quart bowl of a small household electric mixer and warmed to a temperature of 86°F.(30°C.), then whipped at medium speed for 30 seconds. The cream of tartar was added, and the whipping continued for 60 seconds; the sugar was added gradually over a period of 1 minute and the whipping continued until the meringue formed a soft peak when the beater was lifted out. The temperature and specific gravity of the meringue were taken and recorded. The egg yolks and water (10 ml. for the fresh eggs and sirup treated yolks, 15 ml. for the other treatments) were beaten at medium speed in a second bowl for 5 minutes; the temperature and specific gravity of the foam were recorded. The beaten yolks were carefully combined at low speed with the meringue from the whites, and the specific gravity of this mixture was recorded. Half of the thrice-sifted flour was added to the egg mixture and incorporated by mixing at low speed for 15 seconds; the bowl was scraped with a rubber spatula; the second half of the flour was added and mixed in for 15 seconds; the bowl was again scraped and the mixing continued at low speed until the batter had a specific gravity judged to be about 0.38. After the specific gravity and the temperature of the batter were taken, 150 g. of the batter were weighed into a loaf pan (size 4 x 6 inches at the top, 3½ x 5½ inches at the bottom, and 2¾ inches deep) and baked in a preheated oven at 350°F.(176.6°C.) for 30 minutes. Thus, 8 cakes, 1 from each treatment of eggs, were baked the same day, but the order of mixing was varied from day to day.

When the baked cakes were removed from the oven, they were inverted on a rack for cooling, then stored in a closed cabinet overnight. The next day the following measurements were made: the weight of the cake; the volume, determined by seed displacement without removing the cake from the pan *(3)*; the tensile strength of 2 slices, determined by means of an apparatus similar to that used by Pyke and Johnson *(7)*; and the water absorption value *(10)*. Palatability was determined by a panel of 3 judges. The characteristics of the samples judged by the panel were weighted as follows: crumb (evenness of color), 5 points; texture and grain, 25; tenderness, 20; moistness, 15; and flavor, 35. The total score was based on 100 points.

Whole-egg sponge cakes. The formula for the whole-egg sponge cakes was as follows:

Whole egg, white and yolk mixed	96 g.
Sugar, fine granulated	70 g.
Flour, cake	40 g.
Salt	0.5 g.
Cream of tartar	0.5 g.
Water	5-10 ml.

The whole-egg sponge cakes were mixed on a household electric mixer having a 4-quart metal bowl and wire loop whip. The mixer could be operated on 3 different speeds.

The frozen egg samples were defrosted just before they were to be used for making the cakes. A foam was made from the egg, salt (salt was omitted from cakes made from salt treated eggs), sugar, cream of tartar, and water, all of which were placed in the mixer bowl and brought to a temperature of 86°F.(30°C.) before whipping was started. The mixture was whipped at high speed until the specific gravity of the foam was approximately 0.22-0.27. The temperature and specific gravity of the foam were recorded. The flour was slowly added and combined with the foam by mixing at low speed until the specific gravity of the batter was judged to be approximately 0.38. The temperature and the specific gravity of the batter were then taken. The procedures for baking and treatment after baking were the same as described for the separated-egg sponge cakes.

Analyses of variance *(9)* were made for the data on the volumes, tensile strength, and total scores of the cakes made from the frozen eggs.

RESULTS AND DISCUSSION

In Table 1 are presented the average volumes, tensile strength values, and total palatability scores for the cakes made from the separated eggs. In addition to the average total scores for cakes from each treatment and each storage period, there is included the overall average scores for the factors which entered into the total scores for the cakes. The average water absorption values were not significantly different for the various treatments and are not included in the table.

The cakes made from the untreated frozen yolks were the only ones which were of smaller volume than cakes made from fresh shell eggs; likewise, their average volume was significantly lower than that of the cakes made from the eggs treated with salt, sugar, or sirup. The cakes made from eggs treated with the higher level of sugar and the two levels of sirup were significantly larger than those made from the other frozen eggs. The average volume of the cakes made from egg samples stored 6 months was significantly higher than that for the other storage periods.

There was very little difference in the average tensile strength measurements attributable to the treatments given the egg yolks. The average breaking strength values were significantly lower for the 1-week and the 6-month storage periods than for the 2- and the 4-month periods of storage.

The averages of the total scores for the cakes given by the panel of three judges indicated that the cakes made from the untreated frozen yolks were least desirable. These cakes scored low on crumb because small yellow particles were visible; they also scored low on the factors of moistness, tenderness, and flavor. The cakes made from the egg yolks treated with the higher level of salt had the lowest score of all for flavor; they were considered too salty in taste. The total scores for the cakes from the sugar and sirup treated yolks were significantly higher than for cakes from yolks given other treatments. The storage periods affected the total scores only to a slight degree.

In making the cake batters it was found that the thick, gummy, untreated yolks did not disperse readily. This finding is in agreement with that reported by Pyke and Johnson *(8)*.

In Table 2 are given the average volumes, tensile strength values, and total palatability scores for the cakes made from the whole mixed eggs; also, the overall scores on the factors which entered into the total scores

TABLE 1

Average volumes, tensile strength values, and judges' scores for sponge cakes made from separated fresh eggs and frozen eggs the yolks of which were given selected treatments before freezing and storage at about 0°F.(—18°C.)

Period of storage of frozen eggs	Fresh shell eggs	Nothing added	Treatments of frozen yolks						Average for frozen egg cakes
			Salt		Sugar		Sirup		
			Teaspoons[c]		Tablespoons[c]		Tablespoons[c]		
			1	2	1	2	1	2	
Average volumes[d] of baked cakes per 100 grams—ml.									
1 week	564	543	582	591	581	583	587	578	578
2 months	542	520	572	570	564	582	587	603	571
4 months	564	534	573	569	552	595	590	603	574
6 months	576	540	589	588	610	603	602	619	593
Average for all storage periods	561	534	579	580	577	591	592	601	
Average tensile strength,[d] grams per sq. cm.									
1 week	35.7	33.9	37.9	33.3	34.9	30.6	32.7	35.1	34.1
2 months	41.3	40.2	35.6	39.7	38.7	37.5	36.8	34.7	37.6
4 months	34.7	38.2	35.9	38.2	43.0	36.0	34.5	33.3	37.0
6 months	32.7	37.0	35.0	33.2	32.5	34.6	36.5	31.2	34.3
Average for all storage periods	36.1	37.4	36.1	36.1	37.3	34.7	35.1	33.6	
Average total scores[d] given by palatability panel									
1 week	85.1	80.3	84.6	83.1	87.3	88.1	86.7	87.5	85.4
2 months	86.1	77.9	88.1	84.9	88.6	90.2	90.2	90.4	87.2
4 months	89.2	78.1	85.6	80.4	88.2	89.1	91.2	89.5	86.0
6 months	89.5	78.6	85.4	77.8	89.8	91.8	91.7	92.3	86.8
Average for all storage periods	87.5	78.7	85.9	81.6	88.5	89.8	89.9	89.9	
Factors judged	Overall average of scores for the 5 factors judged by the palatability panel								
Crumb 5	4.9	2.8	4.4	4.1	4.8	4.8	4.8	4.9	
Texture and grain 25	20.2	18.9	20.7	20.5	20.7	20.6	21.1	20.5	
Tenderness 20	17.2	15.2	16.4	15.9	17.1	18.0	17.8	18.3	
Moistness 15	12.4	10.9	12.1	11.4	12.8	13.1	13.0	13.1	
Flavor 35	32.8	30.9	32.3	29.6	33.1	33.3	33.2	33.1	

[c] The weight of added ingredients per cup (243 g.) of egg yolk were: salt, 6.0 and 12.0 g.; sugar, 12.5 and 25.0 g.; sirup, 20.5 and 41.0 g. In percentage these values are approximately thus: salt, 2.5 and 5.0%; sugar, 5.0 and 10.0%; sirup, 8.4 and 16.8%.

[d] The least differences in means necessary for significance are as follows:

		Volume	Tensile strength	Total scores
For treatments,	5% point	12.8	3.01	1.45
	1% point	17.1	4.10	1.91
For storage,	5% point	9.7	2.34	1.10
	1% point	12.9	3.09	1.45

for the cakes. With the exception of the fresh-egg cakes and those made from the eggs treated with the higher level of salt, the volumes were greater than for corresponding cakes made from the separated eggs.

The pretreatments of the frozen whole eggs influenced some of the characteristics of cakes made from them. The cakes made from the untreated frozen eggs were somewhat larger in volume but similar in palatability to the cakes made from fresh shell eggs. Those made from the eggs treated with the higher level of salt were significantly smaller in volume and lower in palatability score than the other cakes; they were the least acceptable in flavor, tenderness, and moistness. The sugar and sirup treated eggs produced cakes significantly larger in volume and higher in palatability scores than did the other eggs. The cakes made from eggs treated with the higher level of sirup had the lowest tensile strength value, an indication of greatest tenderness; those made with the higher level of salt were least tender.

The length of the storage period for the eggs seemed to have no consistent effect upon the quality of the cakes, as may be observed from the data in Table 2.

The cake batters made from the frozen whole eggs differed in their characteristics. The whole egg magma treated with the higher level of salt produced a foam similar in specific gravity to that yielded by the other treated eggs, but, as the flour was added, the foam immediately began to break, and by the time the flour was incorporated, the batter was thin and of a relatively high specific gravity, a value of 0.44 as contrasted with 0.37 to 0.39 for the other batters. On the other hand, the foams from the sugar and sirup treated eggs were of fine texture and seemingly more stable; it required several seconds to reduce the batters to the desired specific gravity after the flour was added; the batters were very viscous.

The baked cakes differed in appearance. The cakes made from the salt treated and untreated eggs were usually flat or slightly rounded on top; those made from the sugar and sirup treated eggs usually sagged more or less in the center; the same condition was occasionally observed in cakes made from fresh shell eggs. The crusts of the cakes made from the sugar and sirup treated eggs were of fine grain and often somewhat darker in color and less glossy than the crusts of the other cakes.

Since in this study the amount of sugar in the sponge cake formulas was not reduced to compensate for that added to the treated eggs, it may be that the increase in volumes and palatability scores of the cakes made from yolks or whole eggs treated with sugar or sirup resulted from the presence of the extra sugar in the cakes; this possibility needs further study.

SUMMARY

Egg yolks and whole egg magma, untreated and treated with two levels each of salt, sugar, and white corn sirup were frozen and stored in a home-type freezer at a temperature of about 0°F.(−18°C.). Samples were withdrawn from storage at the end of 1 week and after 2, 4, and 6 months for testing their culinary value in sponge cakes. The yolks were used with untreated frozen whites.

TABLE 2

Average volumes, tensile strength values, and judges' scores for sponge cakes made from fresh eggs and frozen whole eggs given selected treatments and stored for periods up to six months at about 0°F.(—18°C.)

Period of storage of frozen eggs	Fresh shell eggs	Nothing added	Treatments of frozen eggs						Average for frozen egg cakes
			Salt		Sugar		Sirup		
			Teaspoons[a]		Tablespoons[a]		Tablespoons[a]		
			1	2	1	2	1	2	
Average volumes[f] of baked cakes per 100 grams—ml.									
1 week	561	611	600	528	621	627	647	654	613
2 months	562	595	591	530	617	634	635	644	607
4 months	548	618	615	573	634	638	644	648	624
6 months	562	606	628	590	647	652	654	654	623
Average for all storage periods	558	607	608	555	630	638	645	650	
Average tensile strength,[f] grams per sq. cm.									
1 week	41.1	38.8	37.6	45.0	32.4	34.6	33.2	28.9	35.8
2 months	36.0	36.3	33.0	40.2	33.6	31.5	33.8	25.6	33.4
4 months	39.0	35.6	36.0	40.0	35.5	30.9	33.3	30.7	34.6
6 months	39.4	34.7	33.9	33.6	33.5	34.3	30.3	29.4	32.8
Average for all storage periods	38.9	36.3	35.1	39.7	33.7	32.8	32.7	28.7	
Average total scores[f] given by palatability panel									
1 week	87.2	88.2	85.3	74.3	89.9	90.4	90.9	90.3	87.1
2 months	90.3	89.4	87.4	75.3	90.9	91.8	91.1	90.4	88.1
4 months	88.1	89.9	86.2	76.4	91.9	92.2	90.7	91.5	88.3
6 months	90.2	86.0	78.9	70.4	91.8	93.2	90.4	90.0	85.9
Average for all storage periods	88.9	88.4	84.4	74.1	90.9	91.9	91.0	90.5	
Factors judged	Overall average of scores for the 5 factors judged by the palatability panel								
Crumb 5	4.8	4.7	4.7	4.6	4.9	4.9	4.9	4.9	
Texture and grain 25	21.2	21.8	22.0	20.3	21.9	22.4	22.4	21.8	
Tenderness 20	16.7	16.8	16.1	14.8	17.6	17.9	17.3	17.6	
Moistness 15	12.8	12.3	11.8	10.8	13.2	13.4	13.0	12.9	
Flavor 35	33.4	32.8	29.8	23.6	33.3	33.3	33.4	33.3	

[a] The weight of added ingredients per cup (243 g.) of whole egg magma were: salt, 6.0 and 12.0 g.; sugar, 12.5 and 25.0 g.; sirup, 20.5 and 41.0 g. In percentage these values are approximately thus: salt, 2.5 and 5.0%; sugar, 5.0 and 10.0%; sirup, 8.4 and 16.8%.

[f] The least differences in means necessary for significance are as follows:

		Volume	Tensile strength	Total scores
For treatments,	5% point	7.9	2.71	1.43
	1% point	10.4	3.57	1.88
For storage,	5% point	5.9	2.04	1.06
	1% point	7.9	2.69	1.40

Under the conditions of this study, untreated, frozen egg yolks were not desirable for use in sponge cakes, but untreated whole egg magma produced sponge cakes of good quality. Egg yolks treated with salt in the proportion of 2 teaspoons per cup (about 5% salt) produced cakes too salty in flavor; and whole egg magma treated with this level of salt produced cakes low both in volume and in palatability. Eggs treated with sugar or sirup (either 1 or 2 tablespoons per cup) produced cakes of greater volume and higher palatability score than untreated or salt treated eggs.

The length of the storage period was not as important a factor as the treatment of the eggs upon the leavening property of eggs frozen and stored for periods up to 6 months.

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THE EFFECT OF ASCORBIC ACID ON THE OXIDATION OF HEMOGLOBIN AND THE FORMATION OF NITRIC OXIDE HEMOGLOBIN[a]

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(Manuscript received June 9, 1951)

In previous work from this laboratory *(1)* it was found that the addition of ascorbic acid to hemoglobin solutions in the absence of nitrite resulted in rapid discoloration and greening, whereas in the presence of nitrite the ascorbic acid brought about fixation of nitrite, producing a comparatively permanent "cured meat" color. Because of the possible importance of these reactions to problems of color retention in meat, the literature on this subject has been reviewed and further experiments are here reported.

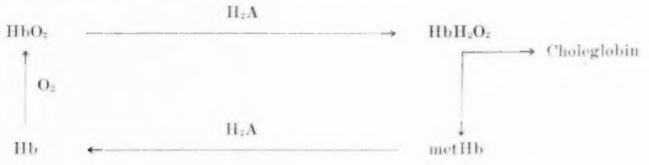
The known reactions of the hemoglobin pigments with ascorbic acid are as follows:

1. Reduced hemoglobin (Hb), purplish red in color, does not react with ascorbic acid (H_2A) in the absence of oxygen.

2. Oxyhemoglobin (HbO_2), bright red in color, undergoes a series of reactions with ascorbic acid *(1)*. The latter donates hydrogen, resulting in an unstable hydrogen peroxide derivative of hemoglobin (HbH_2O_2). The subsequent oxidation of the hemoglobin molecule proceeds in 2 directions. A relatively small portion is converted to a green decomposition product, choleglobin, which eventually precipitates as a green hemochromogen. The remaining HbH_2O_2 oxidizes to methemoglobin (metHb). The reaction may be expressed as follows:

3. Methemoglobin, the ferric hemoglobin, brownish in color, is reduced by ascorbic acid to Hb, the original ferrous hemoglobin *(3)*, which in the presence of oxygen becomes bright red HbO_2.

Reactions 2 and 3 can go on simultaneously. In fact, Lemberg and Legge *(4)* postulate a continuation of the cycle:



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The choleglobin concentration builds up as the cycle repeats itself. This work by Lemberg and Legge as well as the observations on reduction of metHb *(3)* were carried out under physiological conditions of temperature, pH, etc. The reactions have not been studied under meat storage conditions.

4. Nitric oxide hemoglobin (NOHb), the bright pink, heat stable pigment of cured meats, is readily formed in the presence of ascorbic acid and nitrite *(1)*. This reaction has not been thoroughly studied. Presumably NOHb, like COHb *(6)* is incapable of forming a hydrogen peroxide derivative with hydrogen donors such as ascorbic acid, and is therefore not oxidized to metHb and choleglobin by ascorbic acid (although both NOHb and COHb are rapidly oxidized by the direct addition of hydrogen peroxide). Under these conditions the reducing action of ascorbic acid on metHb would be expected to predominate. This is important both in color fixation (since nitric oxide reacts with Hb but not with metHb) and also in protection of cured meat color, since NOHb is readily oxidized to metHb *(7)*.

The experiments reported in the present paper extend the observations on ascorbic acid and the hemoglobin pigments over a range of temperatures, concentrations, and pH values of interest in meat preservation.

METHODS

Preparation of hemoglobin. The hemoglobin solutions used in these experiments were prepared from washed red cells from defibrinated hog or beef blood. The cells were laked with 4 parts of distilled water and stored in a freezer at −20°C. At the time of use the pH was adjusted to 5.6 with HCl and the stroma material filtered off before final dilution to the desired concentration.

Total hemoglobin in the diluted solutions was measured by the method of Evelyn and Malloy *(2)*. The method consists of an initial oxidation of all the hemoglobin pigments present to the ferric state with ferricyanide, followed by formation of methemoglobin cyanide, the absorption of which is read in the Evelyn photoelectric colorimeter using a 540-mμ filter. Values for total hemoglobin by this method did not change much even when choleglobin formation was far advanced and the solutions were green in color, provided that precipitation had not occurred.

Oxidative discoloration of hemoglobin solutions. The oxidation of hemoglobin under the conditions of these experiments resulted in a mixture of metHB, ferrous and ferric choleglobins, and possibly other oxidation products. The method proposed for the quantitative analysis of such complex mixtures *(5)* was considered too involved for the rapid analysis of a large number of samples. A simpler test was therefore devised which would indicate the approximate extent of discoloration or loss of red color.

Both HbO_2 and NOHb absorb strongly in the 540-mμ region; neither shows peaks or high general absorption between 600 and 700 mμ. The oxidation products of hemoglobin, on the other hand, absorb much more strongly in the latter region. MetHb has a pronounced peak at 630-635 mμ, ferrous choleglobin at 628 mμ, and ferric choleglobin at 670 mμ *(4)*.

It was found that percent transmission readings on an Evelyn photoelectric colorimeter with a 635-mμ filter set at 100% transmission with distilled water were a useful index to the visual appearance of hemoglobin solutions. The values, of course, are not specifically related to any particular compound; all of the oxidation products mentioned contribute to absorption in this region. Choleglobin absorbs to a greater extent than metHb. Under the rather extreme set of conditions where 2 samples in the same experiment oxidize along different pathways, the one in which the oxidized product is largely choleglobin will appear to retain more red color than the sample oxidized mainly to metHb when both have the same percent transmission at 635 mμ. In general, however, the transmission readings of such oxidizing solutions parallel rather closely the retention of red color as observed visually. The transmission readings are used throughout this

study to indicate both loss of red color through hemoglobin oxidation, with a consequent drop in transmission, and color fixation in nitrited hemoglobin solutions, which is accompanied by a reduction of metHb and increase in transmissions.

Taken in conjunction with metHb values, the transmission readings at 635 mμ give an indication of the extent of choleglobin formation. That is, a fall in transmission, accompanied by constant or diminishing metHb, is assumed to be due largely to formation of choleglobin.

MetHb was measured by the method of Evelyn and Malloy *(2)*. Since an initial reading at 635 mμ is used in this method, the same solutions used for the transmission values could then be converted to the cyanide derivative for metHb determinations, by the addition of a drop each of 12% acetic acid and 5% KCN to 10 ml. of the hemoglobin preparations in colorimeter tubes. The formula for calculation of metHb concentration, after revision of the Evelyn and Malloy constant for differences in dilution, becomes:

MetHb, % equals $(L_1 - L_2)/2.77$, where L_1 and L_2 are optical densities at 635 mμ before and after converting to methemoglobin cyanide.

All hemoglobin experiments were carried out in 0.02*M*. phosphate buffer, pH 5.6. Ascorbic acid was adjusted to this pH before adding to the buffer, or, more conveniently, a mixture of sodium ascorbate and ascorbic acid in the right proportions to give this pH (97 parts salt to 3 parts acid) could be used without preliminary pH adjustment, making due allowance for the lower ascorbic content of the sodium compound.

TABLE 1

Effect of temperature and concentration of ascorbic acid on oxidation of hemoglobin solutions [a]

Time	Temperature	H_2A concentration	Methemoglobin as % of total Hb (Initial is 13)	% tranmission at 635 mμ (Initial is 65)	Visual appearance
hours	°C.	%			
1	40	0	32	47	dull red
		0.02	15	47	dull red
		0.10	1.2	20	greenish purple
		0.50	0.4	16	greenish purple
24	24	0	39	42	brownish red
		0.02	9.7	64	bright red
		0.10	9.3	12	green
		0.50	5.7	10	green
24	4	0	21	61	red
		0.02	11	71	bright red
		0.10	10	72	bright red
		0.50	4.6	26	brown purple
24	−20	0	23	58	red
		0.02	30	48	dull red
		0.10	27	45	dull red
		0.50	7.5	21	brown purple

[a] Hb concentration is 0.28%.

RESULTS AND DISCUSSION

Effect of ascorbic acid in the absence of nitrite. Of the variables studied, temperature and concentration of H_2A were of greatest importance in determining both the kind and rate of change. Table 1 summarizes results of an experiment illustrating the effect of both variables.

At 40°C. all concentrations of H_2A used brought about rapid discoloration, the solutions passing through a brownish purple stage, then turning green, followed within a few hours by precipitation of the green cholehemochromogen described by Lemberg and Legge *(1)*. The control

samples discolor at a much slower rate and the discoloration is the typical brownish red of metHb. MetHb is reduced very rapidly by higher concentrations of H_2A at this temperature and it is evident that the discolorations obtained with H_2A are largely due to choleglobin formation.

At room temperature, the lowest concentration of H_2A (0.02%) protected the color of the solution. At this concentration, the experimental sample remained better than the control indefinitely. The next higher concentration was protected during the first hour, but later turned green. The highest concentration greened very rapidly. No precipitates formed at any concentration at this or lower temperatures with fresh hemoglobin preparations. However, after prolonged freezer preservation of the stock solutions partial denaturation of the proteins seemed to occur and precipitates did form at room temperature.

In the refrigerator, both 0.02 and 0.10% H_2A protected indefinitely as compared to the control. Transmission readings at the end of 24 hours indicate that these samples were not only better than the control samples at this time, but also better than the original hemoglobin solution at the start of the experiment, due to the reduction of some metHb originally present. Choleglobin formation was apparent only at the highest concentration of H_2A.

TABLE 2

Effect of salt on color retention in hemoglobin solutions [a]

Added constituents	Transmission at 635 mμ [b] after 24 hours at		
	24°C.	4°C.	−20°C.
Control	35	57	48
NaCl, 2%	25	50	41
H_2A, 0.1%	5	66	32
NaCl plus H_2A	2	49	28

[a] The Hb concentration was 0.36%.
[b] Initial transmission was 58.

H_2A at all concentrations brought about a discoloration of frozen samples. However, except at the highest concentration, the discoloration was due more to metHb than to choleglobin formation. When the frozen samples were thawed and allowed to stand at room temperature for several hours, transmission of the control continued to decrease, whereas transmission increased in samples containing 0.02% and 0.10% H_2A, so that the latter became distinctly redder than the control. Discoloration with 0.5% H_2A was due to choleglobin and was not reversed on thawing. In this and other experiments to be reported on frozen solutions, it must be remembered that the freezing process not only lowers the temperature but also concentrates all soluble constituents by removal of water as ice.

Evidently the higher the temperature and the higher the concentration of H_2A the greater is the likelihood of discoloration. At lower concentrations and lower temperatures (down to the freezing point) H_2A protects the color.

The addition of 2% salt (C. P. sodium chloride) to hemoglobin solutions brought about more rapid oxidation of the hemoglobin at all temperatures and in the presence and absence of H_2A (Table 2).

Variations of pH within the extreme limits 6.4 to 5.2 of normal meat also affected the rate of oxidation of hemoglobin solutions. The lower the pH the more rapid the fall in transmission at 635 mμ, both in plain hemoglobin solutions and in the presence of H_2A.

Experiments with nitrite. In preliminary work on the development of "cured meat color" with H_2A, it was found that the use of nitrates had no effect on the color under the conditions of these experiments. Nitrates are slowly reduced in meats by bacterial action during prolonged curing processes. This reduction is not accomplished in hemoglobin solutions in the short times necessary to develop the cured meat color with H_2A. For this reason only nitrite was added (as $NaNO_2$) and the concentration selected unless otherwise stated was 0.02%, the upper limit of nitrite allowed in meat. Sugar (dextrose) like nitrate, had little or no effect on formation of NOHb.

The addition of nitrite alone to hemoglobin solutions caused an immediate large drop in transmission and a concurrent increase in metHb. Nitrite is known to bring about metHb formation with both HbO_2 and Hb *(4)*. After the first large drop, transmission continued to drop at a much slower rate over a period of some hours, but the metHb usually decreased. Evidently the oxidation of hemoglobin brought about by nitrite does not stop with metHb.

The initial drop in transmission and increase in metHb were not prevented by adding H_2A prior to or together with the nitrite unless a large concentration (0.5%) of H_2A was used or the pH was lowered. The reduction of metHb by H_2A is a much slower process than the oxidation of hemoglobin by nitrite under most conditions. Full color development could not be attained if the addition of H_2A was delayed until the oxidation of a considerable fraction of the hemoglobin proceeded beyond the stage of metHb.

Figure 1 shows results of a typical experiment in which 0.02% nitrite was added to a hemoglobin solution. Only transmissions are plotted. MetHb concentration increased immediately from 16 to 32%. Addition of 0.5% H_2A at this point resulted in gradual reduction of the metHb over the two-hour period of increasing transmission and formation of NOHb. At the peak of this curve, no metHb remained. The addition of the same concentration of H_2A to another aliquot of the hemoglobin-nitrite solution after it had stood for 2 hours and lost approximately half of its metHb, resulted in the same type of curve, but with much lower peak transmission. Again metHb had completely disappeared when the peak was reached. In subsequent experiments the nitrite and H_2A were added together.

All factors previously found to accelerate oxidation of hemoglobin in the absence of nitrite, accelerated color fixation in the presence of nitrite.

Temperature had a marked effect on the rate of NOHb formation. In the refrigerator color fixation was slow and often incomplete, whereas under the same conditions a more brilliant color developed rapidly at the oven temperature of 45°C. If allowed to remain at this high temperature subsequent fading of the color was also rapid.

Freezing of solutions of hemoglobin and nitrite in the absence of H_2A caused intense oxidation and greening of the solution. However, when

H_2A was present, freezing definitely hastened development of the red color of NOHb.

Salt accelerated NOHb formation and raised the peak of transmission under all conditions. Some effects of temperature, freezing, and the addition of salt on transmission are shown in Table 3.

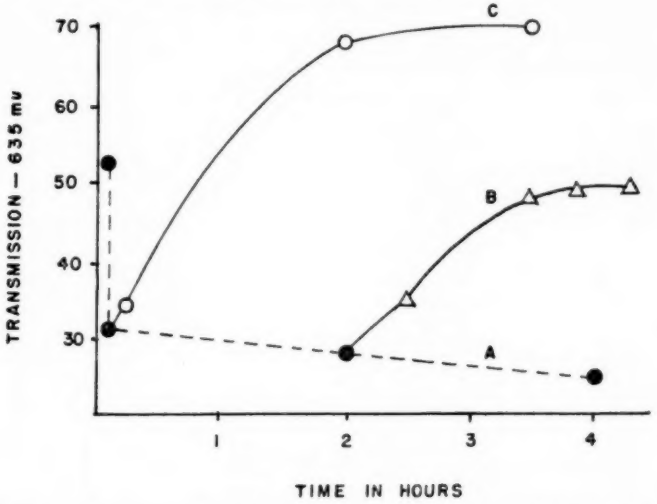

Figure 1. Formation of NOHb on addition of 0.5% H_2A to nitrited hemoglobin solutions at 24°C. Solution contained 0.38% total Hb (of which 16% was metHb) and 0.02% nitrite. A, nitrite alone; B, H_2A added after 2 hours; and C, H_2A added after 5 minutes.

Increasing concentrations of H_2A, at least up to 0.5%, accelerated NOHb formation and gave higher peak values (Figure 2). The lowest concentration of 0.02% was not sufficient to reduce all of the metHb formed by the nitrite. Color fixation was long delayed and very incomplete. With both 0.1% and 0.5% H_2A, metHb was completely reduced, but the reduction took somewhat longer with 0.1% and since some further oxidation products of hemoglobin had formed during this time, the peak was slightly lower. The difference between peak color attained with these 2 concentrations of H_2A was too small to be detected visually.

Proper color fixation also seems to be a function of the concentration of hemoglobin and of nitrite; that is, at low concentrations of either, color fixation requires higher concentrations of the other. At hemoglobin concentrations of approximately 0.2% color fixation occurred at nitrite concentrations as low as 0.001% in the presence of 0.1% H_2A. When hemoglobin was further reduced to 0.05%, a higher nitrite level of 0.02% was necessary to secure proper fixation.

Higher hydrogen ion concentrations increased rate and peak of color fixation. At room temperature, with 0.1% H_2A and 0.02% nitrite, the

times required to reach peak color and the highest transmissions attained were as follows: pH 5.2, transmission 76 after 40 minutes; pH 5.8, 68 in 3 hours; pH 6.4, 43 in 7 hours.

TABLE 3

Effect of salt and temperature on development of cured meat color by ascorbic acid [f]

Temperature	Added salt and H_2A	Transmission at 635 mμ [g]	
		2 hours	24 hours
°C.			
45	Control (nitrite only)	24	9
	Salt, 2%	20	precipitate
	H_2A, 0.1%	74	43
	Salt + H_2A	73	45
24	Control	34	17
	Salt, 2%	28	11
	H_2A, 0.1%	51	37
	Salt + H_2A	71	68
4	Control	42	36
	Salt, 2%	37	22
	H_2A, 0.1%	57	53
	Salt + H_2A	67	61
−20	Control	....	8
	Salt, 2%	....	7
	H_2A, 0.1%	....	58
	Salt + H_2A	....	68

[f] All solutions contained initially 0.36% total Hb (of which 15% was metHb) and .02% nitrite.
[g] The initial transmission of the Hb solution, before addition of nitrite, was 58.

Mechanism of NOHb formation with H_2A. The following steps are suggested as the probable mechanism of NOHb formation with nitrite and H_2A, in line with the experimental facts which have been presented. These apply only to color fixation with H_2A, not to the normal meat curing process.

1. HbO_2 is converted to metHb by nitrite. This is probably an essential step in the process, since HbO_2 cannot combine with NO.
2. MetHb is reduced to Hb by H_2A. This is relatively slow as compared to Step 1. The reduction closely parallels color fixation and probably determines the rate of NOHb formation.
3. Hb unites with NO (formed from NO_2 under reducing conditions in an acid medium). This, like Step 1 is a rapid reaction *(7)* and is probably not a limiting factor.

Factors which aid in color development, that is, salt, hydrogen ions, heat, freezing, etc., may do so by catalyzing the reduction of metHb by H_2A but the data presented in this paper are not sufficient to establish this point.

SUMMARY AND CONCLUSIONS

The known reactions of ascorbic acid with heme pigments are reviewed. A simple method is described for following: 1, discoloration due to oxidation in hemoglobin solutions; and 2, development of nitric oxide hemoglobin in nitrited solutions.

In the absence of nitrite, ascorbic acid protects hemoglobin solutions when it is added in low concentrations and at low temperatures. At high concentrations and high temperatures it brings about oxidation and discoloration. Freezing, salt, and low pH values accelerate the oxidation.

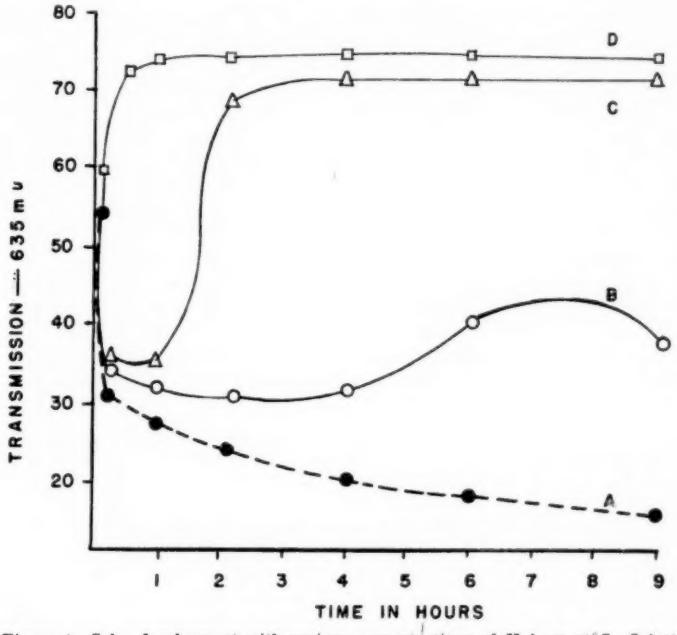

Figure 2. Color development with various concentrations of H_2A at 45°C. Solution contained 0.41% total hemoglobin (of which 11% was metHb) and 0.02% nitrite. A, no H_2A; B, 0.02% H_2A; C, 0.10% H_2A; and D, 0.50% H_2A.

Nitrite brings about a rapid oxidation of oxyhemoglobin to methemoglobin and later to other oxidation products. The addition of ascorbic acid with nitrite causes rapid development of nitric oxide hemoglobin, the cured meat pigment. Nitrates and sugar have no effect on the reaction. Salt, low pH values, high temperatures, and freezing accelerate the color fixation. The possible mechanism is discussed.

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CONSTITUTION OF THE LIPID FROM STORED FLORIDA VALENCIA ORANGE JUICE[a]

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(Manuscript received June 23, 1951)

It has been suggested that some of the off-flavor which develops in canned orange juice during storage may be due to changes which take place in the lipid constituents. Nolte and von Loesecke *(8)* showed that some oxidation of the lipid fraction occurred in canned orange juice during 16 months of storage. Curl *(3)* found in his storage studies of filtered and unfiltered orange juice that the flavor deterioration was less in the filtered juices, from which the suspended material had been removed, and concluded that the suspended material, probably the lipid, must play an important part in the off-flavor which develops on storage. Several investigators have studied the composition of orange juice lipid; but they have not attempted to study quantitatively the changes which take place in the lipid during storage. Matlack *(7)* made a qualitative study of California Valencia orange pulp lipids and Swift *(10)* devised an analytical method for the determination of lipid material in juice.

The lipid from freshly pasteurized Florida Valencia orange juice was studied quantitatively by Swift and Veldhuis *(12)*. A detailed study of the fatty acids from the same juice lipid was made by Swift *(11)*. The purpose of this paper is to present analytical data on the chemical composition of the lipids from another portion of the same lot of pasteurized juice after two years of storage. Also, a comparison is made of the lipid composition of the freshly pasteurized and the stored pasteurized juice.

EXPERIMENTAL METHODS

Processing of juice. The juice had been obtained during the 1947 Valencia orange season from 116 boxes of oranges, mostly packing house rejects. It had been extracted by hand reaming, passed through a commercial finisher, deaerated, pasteurized at about 95°C., and sealed in 46-ounce tin cans. The yield of juice was 1,644 cans of 46-ounce size and 21 cans of 18-ounce size. As the juice was processed it was divided into 2 equal lots; one for the analysis previously reported on the lipids of the freshly canned juice (kept in a refrigerator at 0 to 5°C.); and the other for the present work on the stored juices.

Storage conditions. The canned juice in this study was stored for 24 months under prevailing climatic conditions[c] in the loft of the U. S. Citrus Products Station laboratory.

Extraction of lipid. At the end of the storage period the lipids were extracted following the method of Swift and Veldhuis *(12)* except that an Oliver[d] continuous filter

[a] Report of Study made under Research and Marketing Act of 1946.

[b] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

[c] Annual mean temperature for the year 1947 at the U. S. Citrus Products Station was 73°F.(23°C.). Temperature in loft would probably be slightly higher.

[d] The mention of equipment and products does not imply that they are endorsed or recommended by the Department of Agriculture over other equipment or products having the same or similar properties.

rather than a batch filter was used. The filter drum was first precoated with Hyflo Supercel[4] under a vacuum of about 15 to 20 inches of mercury. When the filter precoat was about 2 inches thick, the drum speed was adjusted to between 25 and 35 r.p.m. and the juice added. In this way about 15 to 25 gallons of juice was filtered per hour, and the lipid removed, along with the precoat, at the rate of about 0.002 inch per revolution.

The material retained by the Hyflo Supercel was extracted with acetone until the color was removed, that is, until the filtrate was about colorless. The acetone was then removed by distillation and the lipid taken up in petroleum ether. This step removed the water that had been extracted from the damp filter cake by the acetone. The petroleum ether-lipid extract was then concentrated to a volume of about 4 liters and stored for about 6 months at 0 to 5°C. until analysis could be started.

Lipid saponification and analysis. The lipid solution was filtered to remove the sterol glycoside that had precipitated, and the petroleum ether was evaporated on a hot water bath. The lipids were then dried by heating on a steam bath under reduced pressure (about 160 mm. mercury absolute) and cooled under a blanket of carbon dioxide. Lipids, 796.85 g., were obtained, which represents a yield of 0.07% based on weight of juice used. The lipid was a dark reddish-brown viscous lipid. When this amount of material was saponified and a detailed analysis made, the breakdown outlined in Figure 1 and the results included in a later section, Table 3, were obtained. No attempt was made to present the state of the constituents as they occurred in the lipid before saponification.

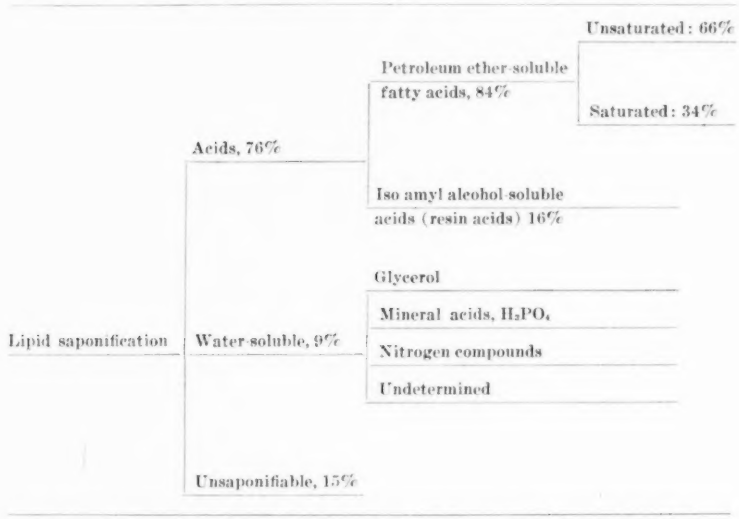

Figure 1. Outline showing approximate distribution into major fractions.

Analytical methods. The unsaponifiable was determined according to the Modified Kerr-Sorber method *(6)* and the fatty acids were determined in the usual manner; that is, the aqueous residue was acidified and the fatty acids were extracted with petroleum ether. The acidified aqueous residue from which the fatty acids had been extracted was made to volume, and an aliquot was extracted with iso-amyl alcohol to obtain the resin acids referred to by Nolte and von Loesecke *(8)*. The glycerol determination was made by a method adapted from the Association of Official Agricultural Chemists (6th Edition, page 551, 1945) on another aliquot of the acidified aqueous solution. Nitrogen was determined on the whole lipid by the Kjeldahl method and the phosphorus by ashing the lipid with magnesium nitrate, precipitating the phosphorus as the ammonium phos-

phomolybdate complex, and subsequent titration with standard alkali. Choline determinations were made on an acid hydrolysate of the lipid using the method of Thornton and Broome *(13)*, but attempts to find choline were unsuccessful. No attempt was made to demonstrate the presence of ethanolamine. The sterol glycoside isolated accumulated at the interface of the aqueous and petroleum ether layers during the extraction of the fatty acids from the bulk of the saponified lipid.

RESULTS AND DISCUSSION

Examination of the Fatty Acids

The bulk of the lipid was saponified and the unsaponifiable matter extracted with ethyl ether in a liquid-liquid extractor. The aqueous residue was then acidified and the fatty acids extracted with petroleum ether again using the liquid-liquid extractor. The fatty acids contained a red-colored constituent similar to that referred to by Swift and Veldhuis *(12)*. Swift and Veldhuis had removed this colored material by esterifying the acids with methyl alcohol and distilling using a Claisen head. To avoid the lengthiness of that procedure, the acids were chromatographed for the removal of color as follows: A sample of the fatty acids containing the colored constituent (having an iodine number 97.67, Wijs 30-min.) was taken up in about 10 ml. petroleum ether. This colored solution containing 5.1204 g. of fatty acid was added to a chromatographic column prepared and standardized according to the method of Fitelson *(5)* as follows: A mixture of 50 parts Hyflo Supercel and 50 parts silicic acid with a small amount of methyl orange indicator, incorporated to make it possible to observe the progress of the chromatogram, was activated and standardized to an activity coefficient of 51.7. The acids were eluted from the column with several combinations of solvents, as shown in Table I.

TABLE 1

Chromatographic separation of colored substance from fatty acids

Fraction No.	Solvent mixture	Fraction collected	Color	Part of total sample
		g.		%
A	20% chloroform, 79% petroleum ether, and 1% ethyl alcohol	4.4915	Very light yellow	87.55
B	3% ethyl alcohol and 97% petroleum ether	0.3311	Light yellow	6.45
C	30% ethyl alcohol and 70% petroleum ether	0.3074	Dark reddish brown	5.99

Since the highly colored constituent had been removed from fractions A and B, these fractions were combined, and the combined fraction, which had an iodine number of 96.85, Wijs 30 min., was examined spectrophotometrically by the method of Brice, Swain, Schaeffer, and Ault *(2)*. Also examined spectrophotometrically were the mixed fatty acids containing the colored constituent. Results obtained in the two spectrophotometric examinations are shown in Table 2.

The long mathematical form given by Brice et al. *(2)* was used in calculating the nonconjugated constituents. It can be seen from the results

in Table 2 that there is very little difference in the composition of the chromatographed acids and the mixed acids containing the colored constituent. Also, the iodine numbers (97.67 and 96.85) agree very well. This seems to be sufficient evidence to warrant the elimination of the chromatographic procedure where spectrophotometric examination is contemplated.

The saturated acids were determined on a sample of the mixed acids by the oxidation method of Fitelson *(5)*. The sample was oxidized for 30 minutes at 70 to 75°C., with performic acid prepared from a mixture of hydrogen peroxide (30%) and formic acid (98-100%). The saturated acids were separated from the reaction mixture according to the method and finally purified by chromatography using dichloromethane as the eluting solvent. The results of this analysis indicated that 30.96% of the mixed fatty acids were saturated acids. The neutral equivalent of the saturated acids was found to be 258.37. No attempt was made to separate the saturated acids into individual constituents since there was very little reason to believe they would be different from those reported by Swift *(11)*.

TABLE 2

Spectrophotometric data on constituents of mixed and chromatographed acids of aged-juice lipids

Constituent	Mixed fatty acids (colored)	Mixed fatty acids (chromatographed)
Conjugated	%	%
Dienes	5.05	4.75
Trienes	0.35	0.39
Tetraenes	0.01	0.005
Non-conjugated		
Arachidonic	0.03	0.00
Linolenic	4.81	5.01
Linoleic	24.28	25.09
Oleic (iodine number)	32.93	31.18
Saturated (by difference)	32.54	33.57

Examination of the Resin Acids

The resin acids etxracted with iso-amyl alcohol were dark reddish brown and very viscous. When the iso-amyl alcohol was removed by heating under reduced pressure, colorless, needle-like crystals were formed in the neck of the flask and arm of the condenser. These crystals were identified as phthalic anhydride by the neutral equivalent, fluorescein test, melting point, and an aniline derivative, phthalanil. Phthalic acid could also be extracted from the resin acids with chloroform, this acid being insoluble in chloroform while other constituents were soluble. Extracted acid was recrystallized from boiling water, 6.4 g. being obtained from 27.0 g. of the resin acids. The phthalic acid represents at least 23% of the resin although this is not to be taken as a quantitative separation. No other constituents of the resin acids have been isolated or identified.

Comparative Analyses of Fresh- and Aged-Juice Lipids

Since the lipid employed in this study was from the same lot of juice used by Swift and Veldhuis *(12)*, except that it was extracted after the

pasteurized juice had aged for 2 years, the results may be compared (Table 3), including also some data from the work of Swift *(11)* on the fatty acid constituents.

TABLE 3

Summary of data from analysis of fresh-pasteurized and aged-pasteurized-juice lipid

Constituent	Fresh-pasteurized[a]	Aged-pasteurized
	%	%
Unsaponifiable	14.81	14.86
Fatty acids (petroleum ether-soluble)[b]	59.00	63.81
Conjugated		
Dienes	0.75	3.03
Trienes	0.092	0.25
Tetraenes	0.096	0.00
Nonconjugated		
Arachidonic	0.00	0.00
Linolenic	5.37	3.20
Linoleic	18.76	16.01
Oleic (iodine number)	17.64	19.90
Saturated (by difference)	16.28	21.42
Resin acids (iso-amyl alcohol soluble)	12.41	12.54
Glyceryl radical CH_2-CH-CH_2	1.53	1.40
Mono acid phosphate radical	4.34	0.40
Nitrogen total	0.61	0.13
Cholinyl radical	2.03	0.00
N calculated as ethanolaminyl radical	1.09	0.47
Sterol glycosides	1.48	1.00

[a] These figures were taken from the work of Swift and Veldhuis *(12)* and Swift *(11)*.
[b] Iodine value, 96.85. Corresponding iodine value of lipid from fresh-pasteurized juice was 112.7.

It is seen that the most striking differences are in the phosphorus and nitrogen contents. Only one-tenth as much phosphorus and one-fifth as much nitrogen was found in the aged-pasteurized-juice lipid as in the fresh-pasteurized-juice lipid. The phosphorus-nitrogen ratio in the lipids of freshly pasteurized juice was 1 to 1, indicating the possible presence of lecithins or cephalins; while in the lipids from aged juice the ratio is 1 to 2. This evidence would indicate that if the phosphatides were present in the fresh-juice lipid a degradation during storage resulted in the loss of nitrogen and phosphorus. The fact that the loss of phosphorus is greater than that of nitrogen is difficult to explain since the ester linkage between the phosphorus and the glycerol is generally considered to be more stable to hydrolysis than that between the phosphorus and choline or other nitrogen constituent. Furthermore, since the nitrogenous constituents of the lecithins or cephalins are generally supposed to be connected through the phosphorus to the rest of the molecule, it is especially difficult to see how nitrogen could be present to a greater extent than phosphorus, unless some sort of recombination such as that reported by Davis and Gill *(4)* occurred between the freed nitrogenous constituents and the remaining parts of the lipid molecule after the phosphorus was lost through hydrolysis. However, this seems highly improbable since the freed constituents in this case are in an excess of aqueous acid solution.

The percent glycerol in the aged-juice lipid was only about 0.5% lower than that of the fresh-juice lipid. Loss of glycerol should be small since

mono- and di-glycerides from the break down of phosphatides are water insoluble and should not be lost in the aqueous phase.

About 5.0% more fatty acids were found in the aged-juice lipid than was reported for the lipid from freshly pasteurized juice. This may be a result of the decomposition of the phosphatides.

When the data obtained from the spectrophotometric examination of the fatty acids from the aged-juice lipid (Table 2) are compared with those obtained on the freshly canned-juice lipid (Table 3), some changes are evident. In general, the percentages of diene and triene acids have increased in the aged-juice lipid while the percentages of linolenic and linoleic acids have decreased. This may be taken as probable evidence of oxidation. Some investigators *(1, 9)* have observed increases in conjugation as a result of oxidation under somewhat different experimental conditions.

The acids from pasteurized-aged-juice lipid contained a higher percentage of saturated and oleic acids than did those from pasteurized-fresh-juice lipid. This was probably because of the loss of the more unsaturated acids. If the oxidation of the unsaturated acids progressed to the point of forming shorter chained fatty acids, they would be of 9 C atoms or less, and consequently would be lost in the aqueous phase which in the juice is present in great excess. Formation of saturated acids from unsaturated acids by reduction is not likely. It is true that the percent saturated acids has increased during storage, but this does not mean that those constituents have changed. The saturated acids are generally considered to be stable and changes in those originally present during storage would not be expected. The differences in the values obtained for the saturated acids by the spectrophotometric and the Fitelson oxidation methods may be due to the presence of hydroxy acids formed by the partial oxidation of the unsaturated acids.

Whereas phthalic acid was isolated and identified in the resin acid fraction from the aged-juice lipid, phthalic acid was not found when a sample of the resin acid from pasteurized-fresh-juice lipid was re-examined.

Lipid Changes in Relation to Juice

It has been observed in this laboratory that the removal of lipids by filtration from orange juice is accompanied by a loss of most of the characteristic flavor. While part of this may be due to the simultaneous removal of peel oil, it seems likely that there is a relationship between taste and the lipids and that changes in amount or composition of these substances would be reflected in the flavor. In the present case it appears that changes in composition on storage also result in a decrease in amount of lipid as isolated and that split products, being more water-soluble than the original lipid, are transferred to the aqueous phase. Here they may have a direct effect on the flavor of the stored juice or may react with other water-soluble constituents to produce new substances of bad flavor. That such a loss occurs is supported by the yields obtained in the present study and those obtained in the same amount (1,100 kg.) of the same juice by Swift and Veldhuis *(12)* that had not undergone storage. These results were, respectively, 797 and 1,000 g. More recent analytical data on other juices bear

out this conclusion. It is possible to calculate the amounts of the components still in the lipid phase and to infer something about the amounts of them that must have escaped into the aqueous phase. For this purpose, the amounts of lipid isolated and the percentage of each component were employed. Results of this calculation are presented in Table 4.

TABLE 4

Amount of constituents obtained from equal amounts (1100 kg.) of juice

Constituent	Pasteurized-fresh	Pasteurized-aged
	g.	*g.*
Total lipid	1000.0	797.0
Unsaponifiable	148.1	118.4
Fatty acids (petroleum ether-soluble)	590.0	508.6
Saturated acids	162.8	170.7
Unsaturated acids	427.2	337.9
Resin acids	124.1	100.0
Glycerol	34.3	24.7
Phosphorus	14.0	1.0
Nitrogen	6.1	1.0
Sterol glycosides	14.8	7.9

Assuming the extractions of the juice lipids to be complete with no fractionation occurring during the extraction, and recognizing the limits of the analytical methods used, these data show that in a given amount of juice the following changes occur: 1, a loss of the extractable lipid material into the aqueous phase as a result of storage; 2, the disappearance of unsaturated acids, perhaps as split oxidation products; 3, loss of phosphorus and nitrogen due to the breakdown of the phosphatides (decomposition products of this component, such as ethanolamine, choline, glycerol, glycerol phosphoric acid, and phosphoric acid, are water-soluble and would be lost to the lipid); 4, apparent loss of a part of the unsaponifiable and resin acids for which no explanation is offered; and 5, little change in the saturated acids which are stable and would not be expected to change.

At the present time no explanation is apparent as to why the unsaponifiable matter, the saturated acids, and the resin acids apparently disappear at about the same rate.

SUMMARY

An analysis has been made of the lipids obtained from stored pasteurized Florida Valencia orange juice. During storage, phosphorus has been lost to a greater extent than the nitrogen. The ratio of phosphorus to nitrogen is 1 to 2. Considerable change seems to have taken place in the fatty acids when data are compared with data from fresh-juice lipids. Conjugated acids have increased while nonconjugated acids have decreased. Considerable breakdown occurred in the lipid fraction of the juice; but which components or breakdown products, if any, are responsible for the off-flavor development was not determined. Work is now in progress to demonstrate the effect of these breakdown products on flavor of juice.

The resin acids from the aged-juice lipid contained phthalic acid, but this acid was not found in the freshly pasteurized-juice lipid on re-examination. From the analyses obtained, it would be difficult to predict the state of the lipid after storage.

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THE VARIATION OF THE ASCORBIC ACID CONTENT IN RAW AND PRESERVED CLOUDBERRIES, *RUBUS CHAMAEMORUS* L.

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The cloudberry, the fruit of *Rubus chamaemorus*, grows on bogs in forests and in mountainous terrain. In the unripe stage the fruit is hard and red, while the ripe cloudberry is a soft, red-yellow and later on a yellow berry. *Rubus chamaemorus* has a typical circumpolar extension, and is distributed throughout the northern parts of the great continents surrounding the Arctic Ocean. In the Western Hemisphere cloudberries are thus to be found in Alaska and Canada, and also in certain parts of the White Mountains. A detailed morphological-biological study on *Rubus chamaemorus* was done by Thekla Resvoll in 1929 *(7)*. From ancient times cloudberries have played an important part in the human nutrition as an antiscorbuticum. Even in our days cloudberries are a source of ascorbic acid in the northern parts of Norway, where they may be found in large quantities. The berries are very popular in all of Norway on account of their delicate coloring and delicious taste.

Up to the present time the literature reports on a small number of analyses, concerning the ascorbic acid content of cloudberries. Gøthlin *(4)* showed that cloudberries were as effective as oranges in preventing scurvy in guinea pigs. Nordal *(6)* found that cloudberries contain 90 mg. of ascorbic acid per 100 g. of fresh material, and Schulerud *(8)* states the value of ascorbic acid to be 80 mg. per 100 g. These values were determined by direct titration. Erkama *(3)* found 150 mg. per 100 g., using a colorimetric method with dichlorophenol-indophenol. Other Finnish workers, cited by Erkama, found 88 mg. per 100 g. (Virtanen, 1932), and 90 mg. per 100 g. (Simola, 1945).

In 1947 and 1948 extensive work on cloudberries was done at the State Research Institute of Home Economics, Stabekk, to determine the amounts of ascorbic acid at different stages in the ripening process. Berries in the hard, red stage were also picked and kept in-doors for ripening, as this procedure is commonly used in the Scandinavian countries. In order to get the cloudberries as fresh as possible, a field laboratory was established, in 1947 in Saltdal, Northern Norway, and in 1948 at Sollia, Eastern Norway. Ripe cloudberries were also picked directly into glass jars, and analyzed throughout the winter. Parallel to this work experiments, with material preserved in different ways, were carried out.

PROCEDURE

The ascorbic acid content was determined by titration, using Bessey and King's modification of Tillman's method *(2)*. The content of each glass jar (containing 200 ml.) was thoroughly mixed, and from this mixture 3 samples were analyzed. Samples

of 10 g. were ground in mortars together with 2% metaphosphoric acid, and filled up to 100 ml. with the same solution. After precipitation the clear, reddish extract was titrated with a solution of dichlorophenol-indophenol. The endpoint was distinct.

To control the method, duplicate samples, packed in dry ice, were sent to Dr. E. Brunius at the State Institute of Pubic Health, Stockholm, where the photoelectric method after Bessey *(1)* was used, together with treatment with formalin after Lugg *(5)*.

The titration values, expressed in mg. of ascorbic acid per 100 g. of raw material, are presented in Table 1. As the two methods agree, one may presume that the method here employed is dependable.

TABLE 1

Comparison of the titration and photoelectric methods

Cloudberries	Date	Ascorbic acid, mg. per 100 g.			
		Analyzed at Stabekk		Analyzed by Brunius	
		Direct titration	Reduced by H_2S	Direct titration	Reduced by H_2S
Fermented and frozen	Nov. 3, 1948	103.2	106.1	.....	.....
	Nov. 3, 1948	.....	.....	106.5	111.9
	Nov. 11, 1948	.....	.....	104.3	111.3
Canned sour	Sept. 29, 1948	.....	.....	98.0	103.2
	Sept. 29, 1948	.....	.....	99.6	107.7
	Oct. 7, 1948	93.6	97.0	.....	.....

RESULTS

Fresh Material

Berries analyzed the same day they were picked. Analyses were taken at all stages in the ripening process. The results are presented in Table 2.

There is a considerable difference in the ascorbic acid contents of unripe and of ripe cloudberries. The highest ascorbic acid content was found in ripe, yellow berries. At the stage of over-ripeness the content of ascorbic

TABLE 2

Ascorbic acid contents of freshly picked cloudberries, hulls and leaves

Stage of maturity	Saltdal, 1947			Sollia, 1948		
	Number of analyses	Ascorbic acid, mg. per 100 g.		Number of analyses	Ascorbic acid, mg. per 100 g.	
		Range	Average		Range	Average
Fruits recently set	.....	.....	.....	2	28-30	29
Red-green, hard fruits	.....	.....	.....	1	.....	21
Red, hard fruits, hulls closed	5	13-16	15	13	10-18	13
Red, hard fruits, hulls open	7	16-24	21	7	13-17	15
Red, soft berries, half ripe	.....	.....	.....	4	25-34	30
Ripe berries						
Red	4	69-90	75	.....	.....	.....
Red yellow, small	4	58-75	64	.....	.....	.....
Red yellow, medium	4	53-67	61	76	34-91	63
Red yellow, large	4	40-48	45	.....	.....	.....
Yellow	4	84-118	100	.....	.....	.....
Over ripe, yellow, faded berries	4	67-94	77	.....	.....	.....
Hulls from unripe berries	.....	.....	.....	4	32-66	47
Leaves	.....	.....	.....	6	197-295	248

acid seemed to decrease. The variation of the ascorbic acid content in red-yellow berries was marked.

Cloudberries picked unripe. The ripening process took place in-doors. One part of the berries was kept in the dark, while the other part was ripened in the light. The berries were analyzed as they became ripe, 4 samples of each lot. The results are presented in Table 3.

TABLE 3

Ascorbic acid contents of cloudberries during ripening in-doors

Place and year	Unripe berries. Ascorbic acid, mg. per 100 g.	Approximate temperature during ripening process, °C.	Time of ripening, days	Ripe, red-yellow berries. Ascorbic acid, mg. per 100 g.	
				In light	In dark
Sollia, 1946	49[a]	20	1	91	96
Saltdal, 1947	19	20	2	.....	61
	19	14	2	.....	48
	19	20	4	.....	109
	19	14	4	.....	97
	14	20	4	103	130
	17	20	4	111	.....
	17	20	5	124	.....
	17	20	6	126	.....
Sollia, 1948	10	20	4	77	.....
	13	20	1	48	49
	13	20	2	67	61
	13	20	3	72	69

[a] This sample was picked unripe and sent to Stabekk, where it was analyzed after 3 days. At this time the berries had become soft and lost the hulls.

The light did not seem to influence the amount of ascorbic acid formed during the ripening process. If the ripening, however, took place in a basement at a lower temperature, the increase of ascorbic acid was slower than when the berries were ripened at room temperature.

In the last ripening experiment the moisture content was determined. The material was dried for 4 hours at a temperature of 105°C. The results are presented in Table 4. The variation in moisture content during the ripening process was negligible, and it was found unnecessary to give the results on dry basis.

TABLE 4

Moisture content of cloudberries during ripening

	Unripe berries	Berries ripened out-doors	Berries ripened in doors					
			In light			In dark		
			1 day	2 days	3 days	1 day	2 days	3 days
Moisture, %.....	86.7	87.3	87.5	86.7	87.0	87.5	87.2	86.9

Storage Experiments

Storage experiments were carried out on raw, frozen, boiled, and canned cloudberries. For the series of raw berries, freshly picked material was thoroughly mixed and afterwards put in glass jars, which were filled and

tightly closed. In 1947 the samples were kept in a basement at a temperature of apporximately 10°C. In 1948 the same experiment was carried out consisting of 2 series, of which 1 was kept in a basement at a temperature of approximately 10°C. The other series was kept in cold storage at a temperature of 0-2°C. During the first 2 months analyses were carried out every second week, and later at intervals of approximately 2 months. The content of 1 glass jar was analyzed at each time, and 3 samples were taken from each jar. Parallel analyses were carried out on frozen material after 6 and 8 months of storage.

For the preservation experiments the material was transported in barrels from the picking place to Stabekk. For this reason the berries were not freshly picked at the time when the samples were preserved. The frozen berries were kept at a temperature of —20°C. For canning the berries were put in 200-ml. glass jars which were heated to a temperature of 80°C. for 15 minutes. The cooked material was boiled for 5 minutes and kept in glass jars. The surface of the berries was covered with paraffin wax, and the jars were tightly closed. The storage temperature of the canned and of the cooked berries was approximately 10°C. The results are presented in Table 5.

In 1948 the cloudberry crop was small, due to unfortunate weather conditions and an exceptionally dry summer the previous year. The work therefore is not as extensive as originally planned.

SUMMARY AND CONCLUSION

The ascorbic acid content of cloudberries was determined at the picking place in different stages of the ripening process, and under different conditions. Raw and preserved cloudberries were stored and analyzed over a period of 8 months.

A marked increase in titration value was noted as the berries changed from the hard, red stage into a soft, red-yellow stage. The increase occurred even if the berries were ripened in-doors. The titration value also greatly increased in ripe, raw cloudberries at the beginning of the storage period. The 2 series, which had been stored in a basement at approximately 10°C. in 3 weeks reached the increase maxima of 83 and 70% in 1947 and 1948, respectively. In cold storage at 0-2°C., however, about 2 months elapsed before the increase reached its maximum. In the period from October to November the titration values seemed to decrease, but even after 6 months of storage the values were higher than in freshly picked material. When the berries were frozen or heated, no increase occurred during storage.

Photoelectric determination with treatment with formalin showed negligible amounts of unspecified reducing substances. There seems to be an indication that the increasing titration values are due to an increase in the ascorbic acid content of cloudberries. According to the results, one might presume that the formation of ascorbic acid in cloudberries is an enzymic process.

As the titration values decreased, the color of the raw cloudberries changed from red-yellow into yellow. A sharp taste also developed during storage.

TABLE 5

Milligrams of ascorbic acid per 100 g. fresh cloudberries before and during storage [b]

Storage time	Raw, stored at				Frozen, stored at −20°C.					Canned, stored at 10°C.			Cooked, stored at 10°C.	
	10°C.		0-2°C.							Unsweetened		Sweetened	Unsweetened	Sweetened
	1947 I	1948 II	1948 I	1948 II	B 1947 II	B 1947 III	1948 I	1948 II	B 1948 III	1947 III	1947 IV	1948 IV	1948 V	1948 V
Just picked	61	62	79	62	.....	.....	79	62	.....	.....	.....	.....	.....	.....
Before treatment	.....	.....	.....	.....	65	120	105	101	102	120	75	100	90	90
After treatment	.....	.....	.....	.....	.....	.....	.....	.....	.....	109	67	106	86	91
Stored, 1 week	105	101	105	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2 weeks	.....	.....	130	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
3 weeks	111	106	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
4 weeks	.....	.....	134	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
5 weeks	.....	102	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
6 weeks	104	.....	141	.....	64	103	.....	.....	.....	70	.....	.....	.....	.....
2 months	107	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
2½ months	.....	94	142	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
3 months	.....	.....	.....	.....	63	74	.....	.....	102	72	57	89	57	66
4 months	92	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
6 months	82	87	119	91	62	96	93	78	108	91	56	81	52	61
8 months	.....	.....	91	88	.....	100	89	89	90	74	40	68	43	43

[b] All the series from 1947 consisted of material picked in Salidal. In 1948 the material marked I and II was picked at Sollia, while the rest came from Elverum. The canned and the cooked series and the frozen series marked B consisted of material transported in barrels.

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SOME ASPECTS OF MICROBIOLOGY APPLIED TO COMMERCIAL APPLE JUICE PRODUCTION.

PART III. ISOLATION AND IDENTIFICATION OF APPLE JUICE SPOILAGE ORGANISMS

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Over a period of 4 years the authors have carried out an intensive investigation of the microflora of the apple and its juice. A large number of spoilage organisms have been isolated and identified, and observations recorded of their growth characteristics in apple juice and their resistance towards pasteurization. Development of these natural spoilage organisms must be severely restricted if it is the intention to produce a pure natural apple juice of high quality and popular consumer appeal. Particular attention must also be paid to the prevention of contamination from external sources. If the factory microbiologist is familiar with the species of microorganisms indigenous to the fruit supplied to the processing plant, any serious external infection that may develop during production may be speedily detected and the source of contamination more readily located. In this paper we shall describe the spoilage organisms that have been identified on the fruit and in the juice processed at an apple juice factory in Gloucestershire, England. The viability and characteristics of these organisms, when permitted to develop in the juice, and their varying resistance towards pasteurization will be discussed in subsequent papers of this series.

The taxonomy of the genera encountered upon such a basal habitat as the apple presents great difficulty, for during the course of countless routine examinations it is possible to encounter practically the whole of the enormous group of organisms comprising the Eumycees or "true-fungi," and also a not inconsiderable number of bacteria. The diversity of organisms encountered is well illustrated by reference to the publications of other authors. Warcollier *(14)* remarks that in 1889 Kayser isolated from cider 11 yeasts, including both top and bottom varieties, and also species of red-pigmented torulae. Warcollier *(14)* also described the various diseases towards which cider is prone and mentions among several organisms, *Mycoderma vini*, *Mycoderma aceti*, *Bacterium gracile*, and *Bacterium mannitopoeum*. A series of papers on various cider yeasts has been published by Barker *(1, 2)*. Tanner *(10)* has surveyed the work of several investigators on the presence of pathogenic bacteria on fruits and their possible relationship to disease dissemination on fruit offered for sale on Paris street stands. Organisms found included *Penicillium glaucum*, *Rhizopus nigricans*, *Staphylococcus aureus*, *Bacillus termo*, *Bacillus subtilis*, and *Micrococcus candidans*. *Escherichia coli* has also been stated to be always present on apples and other fruits.

Fungal diseases of the apple have received much attention and a fairly extensive literature has been compiled on the pathology of the fruit. The various diseases to which the apple is subject and the fungal causative agents have been described in a publication from the U. S. Department of Agriculture *(13)* which mentions 18 principal organisms together with a list of 21 species responsible for minor rots.

We have found that many of the prevalent organisms causing a pathogenic condition of the fruit do not survive in the expressed juice and consequently we have disregarded such species as spoilage organisms. *Gleosporium album ostrew* may be quoted as a typical example. This attacks the fruit through the lenticels and causes bitter rot, but in no case have we isolated this organism from the juice.

Discrimination must also be made in order to differentiate between those molds which are accidentally transferred to the fruit by contact during handling and processing, and those of normal habitat, which can be responsible for inducing spoilage in the expressed juice. Critical comparative study of organisms arising from or upon the same substratum, if extended over a long period, will enable the plant microbiologist to differentiate those organisms associated with the substratum which can induce greatest spoilage effects from those which are purely innocuous and usually occurring with it.

The organisms that we shall describe, therefore, will be confined to those that have been found present both on the fruit and in the juice and which, if allowed to develop unarrested, are capable of inducing spoilage in the latter.

DESCRIPTION OF THE PLANT

At the factory where this investigation was carried out, apple juice is made by the Boehi process. After temporary storage in silos, the fruit is washed, milled, and pressed. The expressed juice is fined in stainless steel clarification vessels, filtered or centrifuged, and pumped to enamel-lined Boehi pressure tanks where the juice is stored at low temperature and under a pressure of carbon dioxide prior to blending and bottling. A high degree of sanitation is maintained in the plant, all processing equipment being of stainless steel or enamelled metal, and in consequence, the risk of contamination of the juice from external sources has been reduced to a minimum.

METHODS OF ISOLATION AND IDENTIFICATION

Preliminary examination and isolation. In the first instance, direct films for microscopic examination were prepared of organisms detected visually on fruit, pomace, and in various samples of juice obtained from the plant. Subsequently, the fruit or pomace was pulped aseptically in sterile water and after suitable dilution plated out on apple juice agar and incubated for 48 hours at 25°C.(77°F.). The medium was prepared by dilution of apple juice to reduce the sugar content to 1-2%, followed by addition of 0.2% ammonium phosphate and 2% agar. Samples of juice were merely diluted with sterile water before plating out. The size and appearance of the colonies after incubation were recorded, and further films were prepared for microscopic study. Finally, sub-cultures were made on to apple juice agar slopes and freedom from contamination was established microscopically. The slopes were then sealed, allotted a type number and maintained as stock cultures. Thus records were obtained of 1, the

origin of the organism and its appearance prior to culture; and 2, its appearance in culture and during various stages of growth.

Identification. For identification purposes, it was found desirable, although not always essential, to sub-culture from the stock culture on to selected media. Czapek's solution agar was used as a standard medium for identification of the molds in culture. The yeasts were sub-cultured on various media especially suitable for biochemical reactions and fermentation tests as aids to identification. For the bacteria, Blickfeld's solution was utilized. A combination of Gram's staining reaction and the catalase test as suggested by Shimwell *(8)* proved invaluable for placing the latter organisms in their respective groups prior to carrying out more characteristic biochemical tests.

Tabulation of characteristics and adherence to the systematic nomenclature adopted by various authorities proved of considerable aid in identification. Works of reference consulted included Thom and Raper *(12)*, Thom *(11)*, Smith *(9)*, Gwynne-Vaughan and Barnes *(4)*, Guilliermond and Tanner *(5)*, Bergey *(3)*, Jorgensen *(7)*, and Henrici *(6)*. It is hoped to describe elsewhere at a later date, certain species whose identification remained uncertain.

Maintenance of cultures. The majority of the organisms described in this paper have been maintained in stock culture. Many of the molds have been cultured on various media such as soil, and storage at low temperature, etc., but it has also been found desirable to run parallel cultures on apple juice agar to prevent changes in sporulation tendency and segregation of characteristics, in addition to parallel cultrues preserved in 10% sugar solution and by lyophilization.

SPOILAGE ORGANISMS IDENTIFIED

It has not been considered necessary to publish a full description of each organism since in most cases cultural characteristics have conformed to those of the type organism grown on the prescribed media as described in the standard works of reference. The type numbers prefixing the organism described in the following text refer to the isolates which we are preserving in pure culture and which still retain the morphological characteristics of the original strains.

The Mucorales

These organisms were isolated under varying conditions and in some cases two or several cultures of the same organism were obtained. Some exhibited slight strain variations, others displayed no difference and there was a general conformity to type. In general, the characters observed in establishing identification, correspond very closely to those described by Smith *(9)*.

A/71. Mucor mucedo Linne. This organism was frequently found associated with apples, especially with rotten fruit which showed the presence of Penicillium rot. Isolates were obtained from fresh and also processed juice and were common on fermenting pomace. This organism did not remain viable for any length of time in juice stored in bulk under high pressure and low temperatures and was not encountered after 1 or 2 months of storage. It cannot be considered a major spoilage organism if normal precautions are taken for cleansing plant and equipment.

B/21. M. pyriformis Fischer. This organism was frequently isolated from sound and rotten fruit and from juice immediately after pressing. It was not found in juice stored at low temperature and high pressure, but was occasionally isolated from processing equipment, and more frequently from pomace.

T/20. M. racemosus Fresenius. This organism was isolated from apples with partial brown rot and Penicillium rot, damaged fruit, and from the epiderms of apparently sound apples, also on pomace. It was detected in tank-stored juice up to 2 months after filling, after which it did not reappear.

R/31. M. hiemalis Wehmer. This organism frequently occurred at all stages of juice production, but was not found in the storage tanks after 1 month of storage. It was frequently encountered on pomace.

Z/1. Zygorhyncus Moelleri Vuillemin. This species was of widespread prevalence but rapidly disappeared in tank-stored juices and its spoilage qualities were not considered serious. It was often encountered on apples showing *P. expansum* and Sclerotinium rot.

T/2. Rhizopus nigricans Ehrenberg. It was frequently identified on apples, fresh processed juice, and from pomace. It suffered rapid reduction in numbers when the juice was placed in refrigerated pressure tanks and completely disappeared within a few weeks. It was only infrequently encountered on processing equipment and may be disregarded as a spoilage organism if sanitary precautions are taken in processing juice. It was also found associated with rotting fruit but was of inconsiderable prevalence compared with other organisms encountered on damaged and infected fruits.

The Aspergilli

Species of this group were commonly detected during work on the plant, but, although frequently isolated from sound, broken, and moldy fruit, did not contribute in any great degree to the spoilage of apples as received from the growers and on storage of fruit in the silos for periods up to 4 days in very hot weather. These organisms did not appear to induce any extensive deterioration in the fruit.

Strain variations were not marked to any great degree and although variations were encountered, these constituted a very small minority. The majority of the species isolated conformed to the type characteristics of the particular strain isolated. Survival in juice in refrigerated pressure storage was very high, most of the species isolated surviving under such conditions for periods up to 2 years.

Contamination of equipment, surfaces, etc., was not unduly high and may be considered very small indeed when compared to the excessive numbers of penicillia encountered in similar locations.

The following organisms have been identified:

F/2B and Bo/3/16B. Aspergillus niger group. The majority of isolates from this group conformed to two distinct types, which we have been unable to identify and consequently have designated as Strain A and Strain B.

F/41B. A. ustus. The isolates of this group were rare and neither appeared to cause damage to apples in the silo, nor were they of any serious incidence during the whole sequence of processing, in the primary stages of juice storage, or in bottling. Their incidence on the fruit was much lower than anticipated since they are usually found to be the most abundant of all aspergilli in the soil.

A/41C. A. sydowi Bain. and Sart. This organism was isolated from a variety of substrata during apple juice production. Isolates were obtained from apples in the silos, wet pomace, juice at all stages of production, juice in bulk storage for periods of up to 18 months, and also occasionally from bottled pasteurized juice where it existed as heat-resistant spores, but was incapable of development in the presence of low concentrations of carbon dioxide (0.75 volumes at S.T.P.).

Bo/19. A. nidulans Eidam. A large number of *aspergilli* corresponding to the *A. nidulans* group were isolated during processing. They were identified on sound and on molded fruit, juice at all processing stages, juice in storage under high pressure, low temperature for periods of up to 18 months, wet pomace, and on processing equipment. These organisms have also been found in bottles of pasteurized juice where their development was retarded in the presence of low concentrations of carbon dioxide. They did not cause any excessive spoilage of juice under conditions prevalent in the plant.

F/24B. A. wentii group. The characteristics of the organism isolated resembled more closely but were not completely identified with those of *A. alliaceus* Thom and Church. It was found at various stages of processing but diminished in numbers at each stage and was seldom identified in the storage tanks. Its incidence was low on fruit in the silo and it was not viable in pasteurized juice. It may be regarded as possessing little spoilage significance.

R/30A. A. flavipes Bain. and Sart. This organism conformed to type characteristics of *A. flavipes* with the exception that we were unable to observe formation of hülle cells. A limited number only of these organisms was encountered and it is most likely that their presence on the fruit arose from contamination with infected soil. It was found on both sound fruit and fruit infected with *P. expansum* rot, on pomace, and in apple juice at various stages of processing, including juice in bulk storage, and in a small number of instances in pasteurized bottled juice.

R/5. A. ochraceus group. The type characteristics of the organism isolated were very similar to those of *A. ochraceus* Wilhelm. Schlerotia, however, were pink in color, up to 0.7 mm. in diameter, and scattered over the mycelium. This is characteristic of *A. Schlerotium* Huber with the exception that the schlerotia found in our organisms were scanty. In all other characteristics our isolate could be identified with *A. schlerotium* with the exception of the conidia which were globose to sub-globose, echinulate 3.5 μ in long axis, and persistent in chains when mounted, in which respect it conforms to *A. ochraceus* Wilhelm. This organism was frequently isolated from apples in the silo which appeared to be associated, especially as a secondary infective agent in conjunction with *P. expansum* rot and with brown rot *(S. fructigena)*. This was not of great incidence in the processed juice, and was identified only infrequently in tank-stored juice.

AF/3. A. fumigatus group. The characteristics of the strain isolated were similar to those of strain N.R.R.L. No. 163 (Thom No. 118). The incidence of this mold was one of the highest among the Aspergillus series and was encountered throughout all the stages of production, including storage and pasteurized bottled juice. A feature of our own isolate was the production of many deformed heads when grown in apple juice in the presence of low concentrations of carbon dioxide. Cultures of the organism developed well at a temperature of 38-41°C.

A/21B. A. fischeri Wehmer. Isolates were few in number and did not appear often in cultures from juice in storage or from pasteurized juice. Occasional cultures were obtained from fruit in the silos but they appeared mainly to be the result of accidental contamination.

The Penicillia and Related Genera

Study of the penicillia in relation to apples and production of unfermented apple juice shows a great diversity of form and structure but from examination of organisms associated with these substrata it becomes apparent that the penicillia are by far the largest group of organisms (with the exception of the Saccharomycetaceae) to be found in apples and again (excepting the Saccharomycetaceae), are the main cause of spoilage of fruit in storage and in the juice expressed from it.

Normally it is found that yeast fermentation develops rapidly when recently pressed juice is left exposed. After yeast activity has subsided, mold growth develops and in most instances the penicillia are invariably encountered.

Storage of juice at low temperature and high pressure inhibits mold development and may after some time depress the number of viable spores either due to autolysis or to natural degeneration, since a similar phenomenon has been observed in normal cultures that have been maintained for long periods without transfer.

The penicillia are dominative spoilage agents wherever fruit rot occurs, particularly *P. expansum* which is the causative agent of blue mold which

can develop on fruit entirely free from brown rot *(S. fructigena)*. Attack by *P. expansum* results in development of extensive soft rot areas, rapidly spreading until the whole fruit is involved. Conidial masses are reproduced on the rotting area under normal storage conditions as characteristic coremia consisting of massed white conidiophores spreading at the apex to produce a head of penicillate conidia. Subculture on to suitable medium (apple juice agar is recommended since on this medium production of the fasciculate colony is easily obtained) exhibits the typical coremium formation indicated by fascicles of conidiophores, but varying greatly with the particular strain involved.

Regarded from the point of view of production of unfermented apple juice, the genus Penicillium probably constitutes the greatest threat of spoilage. Not only is this group of organisms encountered throughout the whole of processing to the final stages of bottling, but when apples are infected and softened with *P. expansum* rot they are easily crushed when stored in the silo for only a few days with the pressure of merely a shallow layer of fruit above. This crushing of the already softened fruit effects a ready and favorable substratum for rapid yeast development and from the counts made it would appear that one "soft-rot" apple if broken or crushed may contribute more to yeast infection of the juice than several hundred sound unwashed apples.

In the following text only the most frequently encountered strains are considered and the many hundreds of other species encountered have not been included unless they were of sufficiently high incidence to warrant consideration from a processing standpoint.

Bo/3/7C. Penicillium glabrum series. A very large number of species conforming to the characteristics of the *P. glabrum* series have been encountered both on apples and in juice. Isolate *Bo/3/7C* has been selected as representative and resembled closely Culture No. 2467 Thom species *P. frequetans* Westling. This organism was frequently encountered in processed juice. The spores remained dormant in the juice in the storage tanks, undergoing no development nor causing any spoilage during periods of up to at least 18 months.

Bo/3. P. spinulosum Thom. A large number of organisms conforming to the "type" organism were isolated from apples in the silos, juices at varying stages of processing, and from juice in the storage tanks. This organism does not appear to cause spoilage in storage and 0.25 volumes of carbon dioxide at S.T.P. will inhibit conidial germination in bottled juice.

Bo/2. P. cyaneum Bain. and Sart. The "type" culture was isolated from the epidermal washing of an apple. A very few species were isolated from fruit in the silos and occasionally in the processed juice. These species do not appear to cause spoilage in the storage tanks and were only occasionally observed.

16A/1. Brevi-compacta series. A number of organisms isolated from fruit and from the juice at various stages of processing were obtained but we were unable to establish the species. They were identified only occasionally from the juice in the storage tanks and did not appear to be serious from the point of view of spoilage. The presence of 0.25 volumes of carbon dioxide will prevent germination of the spores in bottled apple juice.

Bo/14. P. lanosum Westling. Many strains were obtained conforming to the characteristics of the "type" organism. They were frequently isolated from juice at all stages of production and were persistent contaminants of juice in the storage tanks where the spores remained viable for periods of at least 18 months, but caused no spoilage under the conditions of storage.

Bo/3/5B. P. cyclopium Westling. This mold was repeatedly isolated from apples in the silos, both from epidermal scrapings and washings of apparently sound fruit, and also from fruit showing *P. expansum* rot. It was frequently isolated from bottled juice but conidial growth was completely suppressed in the presence of low concentrations of carbon dioxide.

F/46. Viridicatum group. Organisms conforming to the species of this group were occasionally isolated from apples in the silo but did not appear to be prevalent to any great extent in the juice in bulk storage. The "type" organism was taken from an apple showing extensive *P. expansum* rot. In culture it displayed characteristics common to both *P. viridicatum* Westling and *P. verrucosum* Dierckx.

S/21. P. crustosum Thom. Members of this series were isolated from both sound and rotting fruit and from tank-stored juice for periods of up to 18 months. They did not appear to proliferate in the juice stored in bulk, but grew unrestrictedly in carbon dioxide-free juice.

S/43B. P. expansum. Many organisms were isolated and identified as belonging to the series *P. expansum* (Link) Thom. These were frequently found on rotting fruit and also on wet pomace producing coremia up to 1 cm. in length. Incubation of apples with the strains isolated produced active rots. Growth of these organisms in apple juice is very rapid, giving rise to characteristic colonies which will be described in a later paper.

Bo/6. Paecilomyces varioti Bainier. This organism was found associated with a variety of substrata throughout the stages of processing and the spores remained viable for at least 18 months in tank-stored juice but did not induce any spoilage.

The Fungi Imperfecti

Most of the members of this group which can produce spoilage in apple juice belong to the order Hyphomycetes. We have identified seven species which we consider of sufficient importance to include here.

F1. Fusarium. Species of this organism are difficult to maintain in culture and since the genus is composed of a large number of species, exact identification is difficult. They were frequently found associated with both molded and apparently sound fruit, and grew readily as saprophytes. They have not been isolated from bottled juice and due to the large size of their spores are reduced considerably in numbers after filtration of the juice.

A/T31. Alternaria tenuis (Dem.) pleosporia. This was isolated on many occasions from apples in the silo, from juice at all stages of processing, and from juice in storage tanks. Although encountered in a variety of circumstances it did not appear to possess any spoilage significance in relation to the processing methods in use at the factory.

T/25H. Cladosporium herbarum Link. This organism was frequently isolated from apples, both those showing rot and others visually appearing sound; from processed juice, pomace, and juice in bulk storage for periods up to 2 years.

D/4A. Botrytis cinerea Persoon. This organism occurred frequently, being found on the fruit, pomace, and processed juice. Although its incidence was fairly high, it did not appear during bottling and pasteurization. Although not of serious incidence on apples, it is well able to develop on the fruit and growth may result in loss of sugar and affect the flavor of the juice.

B/37A. Oospora lactis (Fres.). This mold was occasionally encountered on apples and in the storage tanks.

B/39B. Oospora candida. This mold was frequently isolated from apples and freshly expressed juice, but a few weeks of high pressure-cold storage eliminated this organism and hence it was disregarded as a serious contaminant in the juice as processed under the factory conditions. Vigorous fermentation may, however, develop in apple juice at 38-40°C. with production of carbon dioxide and small amounts of alcohol.

The Yeasts

Under this heading we have grouped both the true yeasts or Saccharomyces and the torulae.

Y1-Y7. Saccharomyces. Seven strains were isolated all of which were found at practically every stage of processing. At the time of writing they have not been identified but we hope to describe them in further detail elsewhere.

Y8. Torulopsis. A number of yeasts of the genus *Torulopsis* were encountered in tank-stored juice up to at least 8 months after filling, gradually diminishing in numbers under these conditions.

T/36B, T/37B, and T/38B. Rhodotorulae. Three distinct strains of Rhodotorula were isolated from fruit in the silos, fresh juice, and in juice after filtration and storage. In juice stored under pressure at low temperature they rapidly diminished in numbers and after 8 months their incidence was very low.

Acetic Acid Bacteria (Acetobacter)

RT1. Acetobacter xylinum Brown. This organism was first observed at Gloucester after the 1947 pressing season had been concluded, while routine cultural tests were being made on juice sampled from the storage tanks. It appeared at all stages during processing and also from cultures made directly from apples and thus possessed a high incidence in the plant. It disappeared from tank-stored juice after 6 months.

G6/8. A. oxydans (Henneberg) Bergey et al. This organism was identified in a tank of stored apple juice. It slowly increased in numbers over a period from December to July and then decreased, and 6 months later proved difficult to detect. Throughout the whole period no deleterious effect was noted in the juice and there was no gross change discernible on organoleptic sampling at intervals.

Lactic Acid Bacteria

A large number of lactic acid bacteria are found associated with apples, both on the fresh fruit received from the grower and on fruit in the silos. Freshly expressed juice contains a number of these organisms but after fining and filtration the numbers are reduced.

R/31. Lactobacillus delbrückii (Leichmann) Bijerinck. This bacteria has been isolated from sound apples in the silos, and also from apples infected with blue mold, and with brown rot. It was frequently encountered in freshly pressed juice and juice stored in bulk. It appeared to be of almost universal prevalence during processing.

W/19. L. plantarum (Orla-Jensen). The incidence and habitat of this organism were similar to those of *L. delbrückii*.

R/43. L. leichmanni (Henneberg) Bergey et al. This organism was also prevalent at all stages of processing. It underwent rapid reduction in numbers during tank-storage of juice under pressure and at low temperature.

SUMMARY

In this paper 48 distinct and separate potential spoilage organisms isolated from apples and expressed juice at various stages of processing have been described. These organisms have appeared consistently over a period of 4 years in the plant where the investigation was carried out, and must, therefore, be regarded as peculiar to the fruit grown in the area. We shall discuss in subsequent papers the degree of spoilage that these organisms can impart to apple juice and will also demonstrate the extent to which their relative incidence varies during processing.

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ASCORBIC ACID AND DEHYDROASCORBIC ACID IN SOME RAW AND COOKED PUERTO RICAN STARCHY FOODS[a]

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Although extensive studies have been done in the past on the ascorbic acid and dehydroascorbic acid content of tropical foods by Cravioto, Lockhart, Anderson, Miranda, and Harris *(2)* and Munsell et al. *(4, 5, 6)*, very little information is available regarding the content of these two substances, after cooking, in several starchy foods which are widely used in the dietary of Puerto Rico, as well as of neighboring islands. To fill this gap in our knowledge of these local foodstuffs, the present investigation was undertaken. Samples were tested before and after cooking to determine the combined loss of ascorbic and dehydroascorbic acids during food preparation. The combined ascorbic and dehydroascorbic acids content was determined, because it has been demonstrated by Todhunter, McMillan and Ehmke *(9)*, as well as by DeRitter, Cohen, and Rubin *(3)* that dehydroascorbic acid is as satisfactorily utilized by human beings as the reduced form.

MATERIALS AND METHODS

The samples were obtained at various local markets. The scientific and English names are those from Britton and Wilson *(1)* and Otero, Toro, and Otero *(7)*. The vegetables were cut into 2 equal parts, 1 of which was tested in the raw condition and the other was first cooked and then tested. The vegetables were boiled in water in sufficient quantity just to cover them until done, overcooking being avoided. The container was kept covered during the boiling operation.

The sampling was done by mixing, in a Waring Blendor, the whole weighed half of the material being examined with a known amount of 5% solution of metaphosphoric acid and 10% acetic acid. From the slurries thus obtained, samples were weighed from which aliquots were prepared. The analyses were usually completed within 24 hours of buying and cooking the samples. The method of Roe and Oesterling *(8)* was used for the determination of ascorbic acid and dehydroascorbic acid.

RESULTS AND DISCUSSION

Table 1 was compiled from results obtained after determining the combined ascorbic and dehydroascorbic acids in raw and cooked samples. In this table is included the average percentage loss of these 2 substances during cooking.

A total of 18 different vegetables were assayed. The highest values both for the raw and cooked samples corresponded to the yellow sweet potato (38.8 and 21.9 mg. per 100 g., respectively); cassava, other varieties of sweet potato, arracacha, and the native pumpkin also rated moderately high in this vitamin. On the other hand, "malanga" had the lowest con-

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TABLE 1

Combined content of ascorbic and dehydroascorbic acids of 100 g. portions of raw and cooked starchy vegetables

Common name	Botanical name	Number of samples tested	Combined content of ascorbic and dehydroascorbic acids			Average loss
			Max.	Min.	Average	
			mg. per 100 g.	*mg. per 100 g.*	*mg. per 100 g.*	%
Arracacha (Apio)[b]	*Arracacia xanthorrhiza* Bancroft					
Raw		4	25.1	12.9	19.6	
Cooked		4	14.6	7.8	11.7	40.3
Banana, dwarf Jamaica (Guineo enano)	*Musa Cavendishii* Lamb.					
Raw		5	23.2	16.9	20.7	
Cooked		5	4.7	1.5	3.7	82.1
Breadfruit (Panapén)	*Artocarpus communis* Forst.					
Raw		2	27.4	18.9	23.2	
Cooked		2	13.4	10.2	11.9	48.7
Cassava (Yuca)	*Manihot Manihot* L.					
Raw		7	63.7	19.4	28.5	
Cooked		7	41.6	5.8	16.2	43.2
"Malanga" (Malanga)	*Caladium Colocasia* (L.) W. F. Wight					
Raw		4	4.1	6.3	5.2	
Cooked		4	3.1	1.4	2.0	61.5
"Malango," green (Malango verde)	*Musa sapientum* L.					
Raw		3	29.9	25.2	28.1	
Cooked		3	9.8	4.6	7.6	73.0
"Malango," ripe (Malango maduro)	*Musa sapientum* L.					
Raw		4	13.9	6.2	11.9	
Cooked		4	7.3	3.0	5.0	58.0
Plantain, green (Plátano verde)	*Musa paradisiaca* L.					
Raw		4	21.8	17.2	19.7	
Cooked		4	8.5	3.8	7.3	63.0
Plantain, partly ripe (Plátano pintón)	*Musa paradisiaca* L.					
Raw		4	12.5	7.9	10.1	
Cooked		4	6.4	4.3	5.5	45.6
Plantain, ripe (Plátano maduro)	*Musa paradisiaca* L.					
Raw		4	17.8	5.7	10.9	
Cooked		4	6.9	0.0	3.2	70.6
Pumpkin, native (Calabaza)	*Pepo moschata* (Duch.) Britton					
Raw		5	20.1	6.1	12.5	
Cooked		5	15.0	5.6	9.3	25.6

TABLE 1 (Concluded)

Combined content of ascorbic and dehydroascorbic acids of 100 g. portions of raw and cooked starchy vegetables

Common name	Botanical name	Number of samples tested	Combined content of ascorbic and dehydroascorbic acids			Average loss
			Max.	Min.	Average	
			mg. per 100 g.	*mg. per 100 g.*	*mg. per 100 g.*	%
Sweet Corn root (Lerén)	*Clathea alloura* Aubl.					
Raw		1			6.6	
Cooked		1			2.8	57.6
Sweet Potato, white (Batata blanca)	*Ipomoea Batatas* (L.) Lam.					
Raw		4	33.1	24.6	28.3	
Cooked		4	15.7	7.5	10.9	61.5
Sweet Potato, yellow (Batata amarilla)	*Ipomoea Batatas* (L.) Lam.					
Raw		4	46.0	31.8	38.8	
Cooked		4	26.1	15.9	21.9	43.5
Sweet Potato "mameya," deep yellow color (Batata mameya)	*Ipomoea Batatas* (L.) Lam.					
Raw		4	29.7	18.3	24.0	
Cooked		4	22.7	6.8	12.0	50.0
Taniers, white (Yautía blanca)	*Xanthosoma sagittifolium* L.					
Raw		4	14.2	6.9	9.8	
Cooked		4	4.2	1.6	3.1	68.4
Taniers, yellow (Yautía amarilla)	*Xanthosoma atrovirens* C. Koch & Bouché					
Raw		4	19.5	15.9	17.5	
Cooked		4	9.2	4.1	5.6	68.0
Yam, white (Ñame blanco)	*Dioscorea alata* L.					
Raw		4	12.4	5.5	8.8	
Cooked		4	6.3	4.1	4.6	47.7

[b] Spanish names in parentheses.
[c] Also known as, piche, mafafo, mofongo.

tent, both raw and cooked (5.2 and 2.0 mg. per 100 g., respectively); sweet corn root, white yam, and white taniers also rated very low.

The loss of ascorbic and dehydroascorbic acids during cooking varied from a maximum of 82.1% in the case of the dwarf Jamaica banana to a minimum of 25.6% in the case of the native pumpkin. The mean loss for all the samples tested was 56%. The cooking waters were also assayed, but no ascorbic or dehydroascorbic acid was detected in them.

Those vegetables belonging to the upper group, in servings of 100 g. of cooked product, do supply in the neighborhood of 10 to 30% of the maximum daily adult requirement of ascorbic acid (75 mg. per day). As larger amounts of these vegetables are consumed daily, especially in the rural areas, it has to be concluded that sweet potatoes, cassava, and the other vegetables belonging to this upper group, make a substantial con-

tribution to the ascorbic and dehydroascorbic acids content of the average Puerto Rican diet.

SUMMARY

The combined ascorbic and dehydroascorbic acids content of 18 starchy vegetables commonly used in the Puerto Rican dietary was determined before and after cooking. The average loss of these 2 dietary constituents during cooking was 56%, expressed as ascorbic acid. Those rating highest were all the varieties of sweet potato tested, cassava, arracacha, and the native pumpkin. When cooked they contained between 9 and 22 mg. per 100 g. expressed as ascorbic acid. As appreciable amounts of these vegetables are consumed daily, they contribute, no doubt, a significant quantity of ascorbic and dehydroascorbic acids to the insular dietary.

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EFFECTS OF INTERNAL TEMPERATURE AND OF OVEN TEMPERATURE ON THE COOKING LOSSES AND THE PALATABILITY OF BAKED SALMON STEAKS[a]

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The literature on experimental studies in meat cookery is voluminous, but, to a large extent, the problem of fish cookery has been neglected. Work in this area has been concerned primarily with weight losses during cooking and with the effects of cooking on the nutritive value of the fish.

McCance and Skipp *(5)* determined the percentage losses of weight when catfish, herring, plaice, and whiting were steamed for periods of time varying from 7 to 45 minutes and the percentage losses of weight and of protein when small samples (50 g.) of cod or of pollack were boiled, steamed, or roasted for similar periods. They also studied the nitrogen and mineral losses when herring was either boiled or fried and when catfish was either boiled, steamed, or fried. In the report of their work they give the composition of 64 species of fish which they analyzed in the cooked state. According to Marks and Nilson *(3)*, baking or broiling cod fillets at 375°F.(190.5°C.) for 25 minutes and simmering at 185°F.(85°C.) for 20 minutes had no adverse effect on the nutritive value of the protein. Samples that were overcooked by baking at 450°F.(232°C.) for 35 minutes or by boiling for 20 minutes gave somewhat less satisfactory growth responses. Martinek and Goldbeck *(4)* baked croaker fillets at an oven temperature of 375°F. for 20 minutes and at a temperature of 500°F.(260°C.) for 10 minutes. They reported that the 2 cooking methods caused no differences either in the nutritive value of the protein or in the amounts of thiamine, niacin, or riboflavin in baked fillets. The loss of weight for fillets baked at 375°F.(190.5°C.) was 24.8%, while for those baked at 500°F.(260°C.) the loss of weight was 18.0%. Nilson, Martinek, and Darling *(6)* found no difference in the supplementary value of the protein of broiled [350° to 375°F.(177° to 190.5°C.) for approximately 20 minutes] and pan fried bass fillets.

No attempt was made to control precisely the degree of doneness of the fish or to assess the effects of the different treatments used on the palatability of the cooked product in any of the work referred to above, including the 2 studies in which fish were baked. This may help to account for the fact that directions for baking fish found in recent publications recommend baking temperatures which range from a hot oven or so-called oven broiling method at 500°F. to a moderate 350°F. With these points in mind, the work here reported was undertaken.

Salmon steaks were baked: (1) at 4 different oven temperatures to a constant internal temperature, and (2) to 4 different internal temperatures

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at a constant oven temperature. Cooking losses (evaporation, drip, and total) were determined, press fluid determinations were run on the cooked samples, and the steaks were rated for palatability by a panel of 4 judges.

EXPERIMENTAL PROCEDURE

Preparing the salmon. Fresh Chinook salmon *(Oncorhynchus tschawytscha)* was purchased locally. Then 4 steaks approximately 1 inch in thickness were cut from each salmon from the section immediately anterior to the dorsal fin. After the steaks were cut, the bone was removed and each steak was divided into a right and a left half. The right halves were used for 1 replication, the left halves for a second replication. The 4 slices were designated by the letters A, B, C, and D, in order, beginning with the slice nearest the dorsal fin. The prepared steaks were placed on enameled trays, covered with waxed paper, and placed in a refrigerator until they could be weighed preparatory to baking.

Baking the salmon. The steaks were baked on round aluminum pot roasting racks resting in narrow-rimmed round baking tins. The steaks were baked individually in an oven equipped with a rotating hearth and a window. The steaks in one series were baked at oven temperatures of 350°F.(177°C.), 400°F.(204°C.), 450°F.(232°C.), and 500°F.(260°C.) to an internal temperature of 167°F.(75°C.). The internal temperature was measured by right angled meat thermometers inserted in the steaks. In the second series, the steaks were baked to internal temperatures of 158°F.(70°C.), 167°F., 176°F. (80°C.), and 185°F.(85°C.) at an oven temperature of 400°F.

Design of the experiment.[b] The 4 x 4 latin square design was used in this experiment. The different fish, slices of fish, and temperatures are the rows, columns, and treatments, respectively. Since 2 latin squares were used there were 8 replications for each of the 4 oven temperature treatments.

Slice A, the one nearest the dorsal fin, was baked first, followed by slices B, C, and D, in order, with their assigned treatment. With this procedure as much as 1.5 hours elapsed from the time the first slice (A) was baked until the fourth slice (D) was out of the oven. Thus, the effect of the order of baking the slices was inadvertently confounded with that of the composition of the slices. To eliminate this from the work on variations in internal temperature, a 4 x 4 Graeco-latin square design was used.

Sampling. Upon removal from the oven, the steaks were weighed to determine evaporation, drip, and total weight losses during baking, and samples were taken for the measurement of press fluid. For this determination 2 samples weighing approximately 2.0 g. each were cut from the part of the steak immediately surrounding the bulb of the thermometer. The weighed samples were placed between 2 discs of press cloth and these were placed in aluminum cups with tight fitting lids. These samples were held at 122°F.(50°C.) until the press fluid could be determined.

For the organoleptic tests, 4 samples were cut from each steak, 2 samples from either side of the area around the meat thermometer. The cut samples were placed in covered petri dishes and held at 122°F. until samples from all 4 treatments (oven temperature or internal temperature) were ready to be judged.

Press fluid. The method of measuring the press fluid was a modification *(7)* of that developed by Tannor, Clark, and Hankins *(9)*. Small weighed samples of the fish were pressed between two discs of press cloth for 5 minutes at a temperature of 122°F. and at a pressure of 8,000 p.s.i.g. The difference between the weight of the unpressed sample and the weight of the pressed sample was called press fluid and this is reported as percentage of the unpressed weight.

Organoleptic tests. Each judge ranked samples of steaks from each of the 4 treatments for flakiness, moistness, tenderness, flavor, and over-all desirability. The 4 samples for any one judge came from identical areas of the 4 different steaks. Judges A and B received the first and second samples from the upper part of the dorsal half of the muscle above the thermometer hole. The sample for judge C came from the section between the thermometer hole and the lateral line, that for judge D came from that section of the ventral half of the muscle immediately below the lateral line *(2)*.

[b]Statistical work was done under the direction of Dr. Jerome C. R. Li, Department of Mathematics, Oregon State College.

RESULTS AND DISCUSSION

Baking time. The effects of baking temperature were determined. The time required to bake salmon steaks weighing approximately 7.5 ounces to an internal temperature of 167°F.(75°C.) varied from 16 minutes at 500°F.(260°C.) to 32 minutes at 350°F.(177°C.) (Table 1). The temperature of the steaks when they were placed in the oven was approximately 37°F.(3°C.). Raising the oven temperature from 350°F. to 400°F.(204°C.)

TABLE 1

Effects of oven temperature on length of the baking period for salmon steaks [c]

Oven temperature		Internal temperature			Weight of steaks		Baking time
		Starting		Final			
°F.	*°C.*	*°F.*	*°C.*	*°F.*	*g.*	*ounces*	*minutes*
350	177	38	3.3	167(75°C.)	211.8	7.5	32
400	204	37	2.8	167	220.8	7.8	23
450	232	37	2.8	167	209.0	7.4	18
500	260	36.5	2.5	167	211.3	7.4	16

[c] Based on 8 replications.

reduced the baking time approximately one-third, raising the temperature from 350°F. to 450°F.(232°C.) reduced the baking time almost one-half, while raising it to 500°F. reduced it exactly one-half. Thus, raising the baking temperature from 350°F. to 400°F. caused a greater reduction in baking time than did a comparable increase in oven temperature above 400°F. or above 450°F.

For the determination of effects of internal temperature the steaks in this series weighed approximately 7.75 ounces, and the internal temperature at the start of the cooking period was approximately 43°F.(6°C.). At an oven temperature of 400°F., the average baking time ranged from 21 minutes for steaks baked to an internal temperature of 158°F.(70°C.) to 33 minutes for those baked to an internal temperature of 185°F.(85°C.) (Table 2). The average baking time increased 3 minutes for a 9°F.(5°C.)

TABLE 2

Effects of internal temperature on length of the baking period for salmon steaks [d]

Internal temperature				Oven temperature	Weight of steaks		Baking time
Starting		Final					
°F.	*°C.*	*°F.*	*°C.*	*°F.*	*g.*	*ounces*	*minutes*
43	6	158	70	400(204°C.)	217.9	7.7	21
43	6	167	75	400	224.1	7.9	24
41	5	176	80	400	217.1	7.7	27
43	6	185	85	400	221.1	7.8	33

[d] Based on 4 replications.

rise in internal temperature between 158°F. and 167°F.(75°C.) and between 167°F. and 176°F.(80°C.). To bring about the same rise in internal temperature between 176°F. and 185°F., 6 minutes of additional baking time were required.

Appearance and behavior during baking. On the upper surface of the salmon steaks baked at 350°F.(177°C.) large white lakes of coagulated

protein which had oozed out of the muscle tissue during the baking period detracted from the appearance of the steaks. This phenomena was only slightly noticeable on steaks baked at 400°F.(204°C.). Browning, which did not occur in steaks baked at 350°F., increased as the oven temperature increased and was very decided at 500°F.(260°C.), especially on the cut surface of the dark muscle located just under the skin of the salmon.

At the 2 highest oven temperatures there was considerable spattering of fat on the interior of the oven. Spattering was negligible at 400°F. and none occurred at 350°F.

Cooking losses. The effects of oven temperature on cooking losses were determined. The percentages of evaporation, drip, and total cooking losses from salmon steaks baked to an internal temperature of 167°F.(75°C.) at 4 oven temperatures are given in Table 3. There was no difference in

TABLE 3

Effects of oven temperature on weight losses during the baking of salmon steaks [a] (Internal temperature 167°F.[75°C.])

Oven temperature		Evaporation loss	Drip loss	Total cooking loss
°F.	°C.	%	%	%
350	177	12.9	0.6	13.5
400	204	11.9	0.8	12.7
450	232	12.2	0.7	12.9
500	260	13.2	1.2	14.4
Minimum significant difference at the 5% level		.....	0.3	.....

[a] Based on 8 replications.

the losses of weight due to evaporation associated with the 4 oven temperatures used. There was, however, a significant difference in both evaporation and dripping losses from fish to fish, probably reflecting the wide variation in composition that is known to exist in different fish *(8)*. In addition, there was a significant difference in the dripping losses associated with oven temperature. The minimum significant difference at the 5% level between drip losses at the different oven temperatures is included. An oven temperature of 500°F. (260°C.) caused higher drip losses than the other 3 oven temperatures used. The rendering of the fat, especially from the dark lateral muscle just beneath the skin, was quite noticeable during the baking period in steaks baked at this temperature. Differences in total cooking losses associated with oven temperature were not significant, although total cooking losses did vary from fish to fish. Martinek and Goldbeck *(4)* reported greater weight losses when fish were baked at 375°F.(190.5°C.) for 20 minutes rather than at 500°F. for 10 minutes (24.8% as compared with 18.0%). Their results on weight losses at the 2 oven temperatures are possibly complicated by different degrees of doneness. Assuming they used fillets of comparable size and shape, those baked at 375°F. for 20 minutes were no doubt somewhat more done than those cooked for half that time at 500°F.

The percentages of evaporation, drip, and total cooking losses of salmon steaks baked to 4 internal temperatures at an oven temperature of 400°F. (204°C.) are given (Table 4). The degree of doneness to which the steaks were baked caused a significant difference in the losses of weight due to evaporation and in the total cooking losses, but not in the losses of weight due to drip alone. The minimum significant difference at the 5% level is

TABLE 4

Effects of internal temperature on weight losses during the baking of salmon steaks [f] (Oven temperature 400°F.[204°C.])

Internal temperature		Evaporation loss	Drip loss	Total cooking loss
°F.	°C.	%	%	%
158	70	11.2	0.8	11.9
167	75	13.2	0.9	14.1
176	80	15.1	1.0	16.1
185	85	18.6	1.4	20.0
Minimum significant difference at the 5% level		2.4	.....	2.4

[f] Based on 4 replications.

included. Increasing the internal temperature of the steaks from 158°F. (70°C.) to 167°F.(75°C.) or from 167°F. to 176°F.(80°C.) caused no significant difference in losses of weight due to evaporation or in total cooking losses. However, increasing the internal temperature from 176°F. to 185°F. (85°C.) caused a significant increase in both evaporation losses and in total weight losses during cooking. Baking the steaks to an internal temperature of 176°F. resulted in greater evaporation losses and greater total cooking losses than did baking them to an internal temperature of 158°F.

Press fluid. The 4 different oven temperatures used caused no difference in the percentage of press fluid from the baked salmon steaks (Table 5). The internal temperature, however, did result in a significant difference in the amount of press fluid. Steaks cooked to 185°F. yielded less press fluid than those cooked to either 167°F. or 158°F. Steaks heated to 176°F. yielded less press fluid than those cooked to 158°F.

Organoleptic tests. The four judges ranked the samples from each of the 4 treatments in descending order for each of the qualities listed on the

TABLE 5

Press fluid from salmon steaks baked at four oven temperatures (internal temperature 167°F.) and baked to four internal temperatures (oven temperature 400°F.)

Oven temperatures		Press fluid [g]	Internal temperature		Press fluid [h]
°F.	°C.	%	°F.	°C.	%
350	177	65.5	158	70	68.5
400	204	65.6	167	75	67.5
450	232	66.1	176	80	66.4
500	260	65.7	185	85	65.3
Minimum significant difference at the 5% level		.....	.....	.....	1.8

[g] Based on 2 samples from each of 8 replications.
[h] Based on 2 samples from each of 4 replications.

score card. The steaks were ranked for flakiness as opposed to compactness or crumbliness, for moistness, for tenderness, for the development of flavor, and for over-all desirability. The ranks for the steaks were transformed for statistical analysis into normal scores, using Table XX of Fisher and Yates *(1)*. The highest ranking sample received a normal score of 1.03, the second highest a score of 0.30, the third a score of —0.30, while the lowest ranking sample got a score of —1.03.

The average normal scores for steaks baked at 4 different oven temperatures are found in Table 6. Treatment of the transformed scores by analysis of variance revealed no significant differences in any of the 5 palatability characteristics associated with oven temperature.

TABLE 6

Average normal scores for salmon steaks baked at four oven temperatures to an internal temperature of 167°F. (75°C.)[1]

Oven temperatures		Palatability characteristics				
		Flakiness	Moistness	Tenderness	Flavor	Over-all desirability
°F.	°C.					
350	177	—0.05	—0.24	—0.24	—0.01	—0.14
400	204	0.07	0.35	0.14	0.05	0.22
450	232	—0.11	—0.05	0.07	—0.09	—0.14
500	260	0.09	—0.07	0.03	0.06	0.07

[1] Based on 8 scorings by each of 4 judges.

The analysis revealed an interaction between judge and slice in the scores for moistness, flavor, and over-all desirability. There was a significant difference in the way the different judges ranked the 4 oven temperature treatments.

In addition, there was a significant difference in the scores for flakiness associated with the particular slice of the fish. The mean normal scores for flakiness for the different slices follow: slice A, —0.59; B, —0.16; C, 0.28; and D, 0.47. The minimum significant difference at the 5% level was 0.33. Thus, slices C and D were ranked less compact and crumbly than slice B, which in turn was ranked higher in flakiness than slice A. The higher ratings for the flakiness of the slices as they approached the head end of the fish might have been due to increasing fat content of the fish *(8)* or to the fact that slice D was the last to be baked, slice C the next to the last, while slice A, regardless of oven temperature, was always cooked first.

In addition to ranking the salmon steaks, the judges were asked to indicate any samples they considered either overdone or underdone. Out of a total of 32 observations on steaks baked at each oven temperature, steaks baked at 350°F. (177°C.) were considered too done in 5 instances and too raw in 3, those baked at 400°F.(204°C.) were marked too done in 2 instances and too raw in 2 instances, while those baked at 450°F.(232°C.) and 500°F.(260°C.) were considered too raw in 3 instances and too done in 3 instances, each. Thus out of a total of 128 observations of steaks baked to the same internal temperature (167°F.) at 4 different oven temperatures, the judges considered the salmon too done in 13 instances and too raw in 11.

The mean normal scores for salmon steaks baked to 4 different internal temperatures is found in Table 7.

The judges found no difference in the flakiness of the steaks associated with the four internal temperatures studied.

The internal temperature to which the steaks were baked resulted in significant differences in the scores for moistness. With a minimum significant difference at the 5% level of 0.43, steaks cooked to an internal temperature of 158°F.(70°C.) were rated more moist than those from the other three treatments. Steaks cooked to 167°F.(75°C.) were ranked more moist than

TABLE 7

Average normal scores for salmon steaks baked to four internal temperatures at an oven temperature of 400°F.(204°C.) [1]

Internal temperature		Palatability characteristics				
		Flakiness	Moistness	Tenderness	Flavor	Over-all desirability
°F.	°C.					
158	70	0.26	0.80	0.81	−0.64	−0.42
167	75	−0.14	0.04	−0.30	−0.08	0.04
176	80	−0.21	−0.25	−0.09	0.17	0.30
185	85	0.09	−0.59	−0.42	0.55	0.08
Minimum significant difference at the 5% level.....		.....	0.43	.....	0.44	0.44

[1] Based on 4 scorings by each of 4 judges.

those cooked to 185°F.(85°C.). The judges found no difference in moistness between steaks cooked to 167°F. and those cooked to 176°F.(80°C.) or between those cooked to 176°F. and to 185°F. Thus the increase in internal temperature from 158°F. to 167°F. caused the greatest decrease in the score for moistness.

The internal temperature to which the steaks were baked caused no significant difference in the scores for tenderness. There was, however, an interaction between judge and internal temperature.

There was a significant difference in the scores of the steaks for flavor associated with the internal temperature to which the steaks were baked, as well as with the order in which they were baked. With a minimum significant difference at the 5% level of 0.44, steaks baked to an internal temperature of 176°F. or to 185°F. scored higher in flavor than those baked to 158°F. Steaks baked to 185°F. scored higher in flavor than those baked to 167°F. Steaks baked to 167°F. were ranked superior in flavor to steaks baked to 158°F. In addition, the flavor score varied with the order in which the steaks were baked, the last one out of the oven, regardless of internal temperature, scoring significantly higher in flavor than the first three steaks baked.

The internal temperature to which the fish was baked resulted in a significant difference in the scores for over-all desirability. With a minimum significant difference at the 5% level of 0.44, steaks cooked to 158°F. were scored less desirable than those from the other three treatments. The order of baking the steaks also resulted in a significant difference in the scores for over-all desirability, the last steak baked being rated more

desirable than the first 3 out of the oven. Although the internal temperature to which the steaks were baked influenced both flavor and over-all desirability, so did the order of baking.

Out of a total of 16 observations on steaks baked to each of the 4 internal temperatures, those baked to 158°F. were checked as underdone in 8 instances, those baked to 176°F. were considered overdone in 3 instances and underdone in two, while those baked to 185°F. were marked overdone in 5 instances.

SUMMARY

Salmon steaks were baked to an internal temperature of 167°F.(75°C.) at oven temperatures varying from 350°F. (177°C.) to 500°F.(260°C.) and at an oven temperature of 400°F.(204°C.) to internal temperatures varying from 158°F.(70°C.) to 185°F.(85°C.).

Raising the baking temperature from 350°F. to 400°F. shortened the baking time more than did increasing the baking temperature from 400°F. to 500°F. Higher drip losses and excessive spattering occurred when steaks were baked at 500°F. Considerable spattering occurred at 450°F. Oven temperature had no effect on the palatability scores of the baked salmon steaks.

Increasing the internal temperature from 176°F.(80°C.) to 185°F. (85°C.) prolonged the cooking period more and caused greater evaporation and total cooking losses than did a comparable rise in temperature above 158°F.(70°C.) or above 167°F.(75°C.). Steaks baked to 185°F. were ranked higher in flavor but lower in moistness than steaks baked to either 158°F. or 167°F. Steaks baked to 158°F. ranked lower in flavor and in over-all desirability than the other three treatments and were judged underdone. The judges found no differences in the palatability of steaks baked to 167°F. and those baked to 176°F. The last steak out of the oven, regardless of internal temperature, was rated higher in flavor and in desirability than steaks from the other 3 treatments.

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THE B VITAMINS IN RAW AND COOKED LAMB. II. RIBOFLAVIN AND NIACIN

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The methods used in the selection, preparation, and cooking of the lamb reported in the present series of papers has been described by Wilcox and Galloway *(6)*. The same cross breeds were used which were as follows: first and second cross lambs from Columbia ram x range ewe (C_1 and C_2) and from Rambouillet ram x range ewe (R_1 and R_2) and first cross lambs from Targhee ram x C_1 ewes (T_1).

The riboflavin and niacin study on the lamb is reported here. Riboflavin was determined on aliquots from the samples prepared for thiamine analyses by the method of Peterson, Brady, and Shaw *(4)*. The microbiological method outlined in Methods of Vitamin Assay *(5)* was used for the niacin determinations.

RESULTS AND DISCUSSION

The average riboflavin values for raw lamb on the moist basis ranged from 0.202 to 0.265 mg. per 100 g. (Table 1); on the dry, fat-free basis the range was from 1.009 to 1.338. Differences between riboflavin values for the C_1 and R_1 lambs, between years for the lambs or for the C_2, R_2, and T_1 lambs with either type of feeding were too small to be significant. The riboflavin content of different cuts of the grass-fat lambs was similar to that of the same cuts of fed lambs. The variation in riboflavin content of the various cuts of lamb was small with the values for the legs on the dry, fat-free basis being slightly highest. Other workers have reported riboflavin values for lamb on the moist basis of 0.156 mg. per 100 g. in shoulder by Noble, Gordon, and Nelson *(3)*; 0.225 to 0.270 in stew by Cover and Dilsaver *(1)* and McIntire, Schweigert, and Elvehjem *(2)*; and 0.295 in leg by McIntire, Schweigert, and Elvehjem *(2)*.

The average retention of riboflavin in panbroiled chops of the C_1 and R_1 grass-fat lambs was 78.7 and 81.4%, respectively, as compared to 73.8 and 77.8% for the fed lambs when calculated back to the original weight of the muscle (Table 2). Similar retention values were obtained for the roasted legs (88.1 and 76.9% for the grass-fat as compared to 72.0 and 73.2% for the fed lambs). Retention values for both chops and legs were higher on the dry, fat-free basis, however, differences between groups were not significant. Variations between values for either the grass-fat or fed C_1 and R_1 lambs were small. Retention of riboflavin was slightly higher in the cooked chops or legs of the grass-fat lambs than in the corresponding cut of the fed lambs. McIntire, Schweigert, and Elvehjem *(2)* reported riboflavin retentions of 82 and 88% in roast leg of lamb.

The riboflavin content of a serving of one chop averaged 0.08 mg. for both the grass-fat and fed lambs. These chops were 1 inch thick and weighed 145 and 155 g., the average weight of the lean meat being the

TABLE 1

Riboflavin content of raw lamb

	No. of lambs	Chops		Shoulders		Legs		Stew
		Moist basis	Dry fat-free basis	Moist basis	Dry fat-free basis	Moist basis	Dry fat-free basis	Moist basis
		mg. per 100 g.	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*
Grass-fat lambs								
1947 C_1	4	0.218	1.016	0.226	1.375	0.294	1.488	0.220[b]
1948 C_1	4	0.222	1.002	0.208	1.111	0.236	1.188	.....
Average	....	0.220	1.009	0.217	1.243	0.265	1.338	.....
1947 R_1	4	0.243	1.119	0.209	1.126	0.264	1.463	0.221[b]
1948 R_1	4	0.202	0.914	0.218	1.265	0.248	1.231	.....
Average	....	0.222	1.016	0.214	1.196	0.256	1.347	.....
1949 C_2	10	0.246	1.042	.....	.....	0.220[a]	1.140	.....
1949 R_2	10	0.228	0.982	.....	.....	0.222[a]	1.111	.....
1949 T_1	8	0.232	1.021	.....	.....	0.229	1.194	.....
Fed lambs								
1947 C_1	4	0.228	1.070	0.220	1.104	0.244	1.136	0.203[b]
1948 C_1	10	0.221	1.002	0.184	1.209	0.225	1.200	.....
Average	....	0.224	1.036	0.202	1.156	0.234	1.168	.....
1947 R_1	4	0.253	1.182	0.238	1.191	0.261	1.243	0.218[b]
1948 R_1	10	0.227	1.029	0.173	1.018	0.234	1.201	.....
Average	....	0.240	1.106	0.206	1.104	0.248	1.222	.....
1949 C_2	8	0.237	1.060	.....	.....	0.233	1.216	.....
1949 R_2	8	0.248	1.085	.....	.....	0.248	1.211	.....
1949 T_1	8	0.232	1.015	.....	.....	0.227	1.166	.....

[a] 8 animals used for analyses on legs.
[b] 2 animals used for analyses on stew meat.

TABLE 2

Riboflavin content of cooked chops and legs of lamb

	No. of lambs	Moist basis		Dry fat-free basis	
		Cooked meat	Retention[c]	Cooked meat	Retention[c]
		Chops			
		mg. per 100 g.	*%*	*mg. per 100 g.*	*%*
Grass-fat lambs					
1947 C_1	4	0.248	91.4	0.922	90.8
1948 C_1	4	0.219	66.0	0.708	71.0
Average	....	0.234	78.7	0.815	80.9
1947 R_1	4	0.287	84.8	1.024	91.9
1948 R_1	4	0.233	77.9	0.752	83.4
Average	....	0.260	81.4	0.888	87.6
Fed lambs					
1947 C_1	4	0.267	81.6	0.880	82.1
1948 C_1	10	0.238	66.0	0.702	70.8
Average	....	0.252	73.8	0.791	76.4
1947 R_1	4	0.328	88.8	1.072	90.8
1948 R_1	10	0.242	66.8	0.725	70.6
Average	....	0.285	77.8	0.898	80.7
		Legs			
Grass-fat lambs					
1948 C_1	4	0.285	88.1	1.117	94.4
1948 R_1	4	0.265	76.9	0.999	82.2
Fed lambs					
1948 C_1	10	0.264	72.0	0.937	79.1
1948 R_1	10	0.278	73.2	0.943	78.8

[c] Calculated back to original weight of muscle analyzed.

same from both groups of lambs. A serving of roast leg averaging 70 g. contained 0.19 mg. riboflavin for both groups of lambs.

The distribution of riboflavin between the meat and broth in the stews did not follow a definite pattern as it did for the thiamine as reported by Wilcox and Galloway *(6)*. The meat of stew cooked in a small amount of water, with one exception (Table 3), contained more riboflavin than that

TABLE 3

Riboflavin content and retention in cooked lamb stew

	Grass-fat lambs		Fed lambs	
	1947 C_1	1947 R_1	1947 C_1	1947 R_1
In total stew meat, mg.				
Raw	110.12	110.50	101.62	108.88
Cooked				
Small	60.90	78.30	67.10	73.46
Large	62.58	63.62	54.37	65.10
In total broth, mg.				
Small	19.05	21.97	20.69	21.90
Large	28.00	30.78	29.72	24.45
In total stew and broth, mg.				
Small	80.00	100.28	87.79	95.35
Large	90.58	94.45	84.10	89.54
Retention in stew meat, %				
Small	55.4	72.4	67.2	67.0
Large	56.8	58.6	54.4	59.2
Retention in broth, %				
Small	17.3	20.0	20.5	20.4
Large	25.4	28.2	29.5	22.6
Retention in stew meat and broth, %				
Small	72.7	92.4	87.7	87.4
Large	82.2	86.8	83.9	81.8

which was cooked in a large amount of water. Broths from the latter group of stews retained more riboflavin. Retention was calculated as percent of riboflavin in the cooked stew of the amount originally found in the raw stew meat. Total retention in meat and broth was slightly greater when the small volume of water was used for all groups of lambs except for the C_1 grass-fat lambs. However, differences were not significant. Retentions ranged from 72.7 to 92.4% when a small amount of water was used and from 81.8 to 86.8% for the larger amount of water. Similar retentions were obtained in a previous study by Wilcox, Morrell, and Neilson *(7)*. Cover and Dilsaver *(1)* reported retentions of 105 and 123% on the dry, fat-free basis for lamb stews.

As shown in Table 4 the average niacin values for the chops on the moist basis were slightly higher than those for the legs (5.72 to 7.70 mg. per 100 g. as compared to 5.17 to 6.40, respectively); the same was true on the dry, fat-free basis (for the chops 27.2 to 33.9 and the legs 24.8 to 32.0). Values for the shoulders were similar to those for the legs. Differences between the cross breeds of lamb for either year were not significant. McIntire, Schweigert, and Elvehjem *(2)* found 6.3 mg. per 100 g. of niacin in leg and 5.8 in stew meat of lamb.

TABLE 4

Niacin content of raw lamb

	No. of lambs	Chops		Legs		Shoulders	
		Moist basis	Dry fat-free basis	Moist basis	Dry fat-free basis	Moist basis	Dry fat-free basis
		mg. per 100 g.	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*
Grass-fat lambs							
1947 C_1	4	6.42	30.0	.....	.....	.....	.....
1947 R_1	4	5.90	27.2	.....	.....	.....	.....
1949 C_2	10	7.70	32.4	5.97[d]	30.8	.....	.....
1949 R_2	10	7.52	32.4	6.40[d]	32.0	.....	.....
1949 T_1	8	7.64	33.5	5.90	30.7	.....	.....
Fed lambs							
1947 C_1	4	7.23	33.9	5.48	25.6	5.50	27.4
1947 R_1	4	5.72	27.4	5.17	24.8	4.38	21.0
1949 C_2	8	7.12	31.8	5.30	27.6	.....	.....
1949 R_2	8	7.09	31.1	5.74	27.9	.....	.....
1949 T_1	8	7.03	30.7	5.86	30.0	.....	.....

[d] 8 animals used for analyses on legs.

SUMMARY

The riboflavin content of raw and cooked chop, leg, and stew, and raw shoulder of first cross Columbia and Rambouillet grass-fat and fed lambs was studied. Raw chops and legs of second cross Columbia and Rambouillet and first cross Targhee lambs were also analyzed for their riboflavin and niacin content.

Average riboflavin values for the raw lamb ranged from 0.202 to 0.265 mg. per 100 g. Variations between the various cuts of lamb were small.

Differences between riboflavin values for the C_1 and R_1 lambs or for the C_2, R_2, and T_1 lambs with either type of feeding were not significant.

Retention of riboflavin in panbroiled chops and roasted legs was similar for the C_1 and R_1 lambs. Values for the chops varied from 74 to 81% and for the legs, from 72 to 88%. The riboflavin retained after cooking all stews in a small or large amount of water was 85 and 84%, respectively.

The average niacin content for all chops was 6.9 mg. per 100 g. and for the legs, 5.7.

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RETENTION OF THIAMINE AND PANTOTHENIC ACID IN PORK AFTER STEWING

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The term "pork stews" seems strange at first glance but stewed pork is the basis of many popular meat dishes. In some, the meat may be browned, in others not. Temperature of cooking may vary also. These variations in the method of preparation may affect the retention of the B-vitamins. Because pork is one of the best sources of certain of the B-vitamins which are known to be affected by cooking, a study was planned to determine the retention of thiamine and pantothenic acid in pork stews.

EXPERIMENTAL PROCEDURE

Experimental design. The stewing variables were: meat browned and not browned; and three temperatures, pressure-cooked at 15 p.s.i.g., boiled, and simmered. The different combinations made 6 different stewing procedures. Since dishes using stewed pork are usually made with a small amount of water, none of the pork stews were cooked in a large amount of water as had been those for beef and lamb in the previous study by Cover, Dilsaver, and Hays *(2)*.

Lean meat from the shoulder of one hog was specially prepared in small pieces, thoroughly mixed, and enough meat taken for all of the 6 stews and for 2 raw samples. Replications were obtained from other hogs to make a total of 4 series of pork stews.

Meat for stews. The hogs weighed approximately 200 pounds each. The lean meat was cut into approximately 1-inch cubes. The meat from 1 hog was placed in 2 packages, frozen, and stored in a freezer locker until needed. In preparation for the cooking tests the meat from 1 hog was removed on Saturday to the household refrigerator and held at 45-50°F.(7-10°C.) to defrost. The underneath sides of the frozen packages were slashed with a knife and the unopened packages were left on a rack to drain as they defrosted. A pan underneath the rack caught the drippings, which were discarded as is the usual household practice. The meat was defrosted, drained, and in excellent condition for cooking on Monday morning. Both packages were emptied into a dishpan and the meat was mixed thoroughly for 5 minutes. Then 8 500-g. samples of the raw meat were weighed and distributed as follows: 2 raw; 1 brown, pressured; 1 white, pressured; 1 brown, boiled; 1 white, boiled; 1 brown, simmered; and 1 white, simmered.

Standardized cooking procedures. The cooking procedures were standardized in a similar manner to those for beef and lamb in the previous study by Cover, Dilsaver, and Hays *(2)*. All cooking was done in the pressure saucepans even though pressure was used in only 2 of the 6 cooking procedures.

Cooking times were standardized in numerous previous trials. The aim was to produce meat of equal tenderness by all 6 cooking procedures. This result was obtained by adjustments in cooking times. The cooking times selected are given in Table 1. The tenderness of all of the experimental stews was such that the meat neither fell into strings (overcooked) nor was too firm to cut easily with a silver knife (underdone). Within these limits some variation in tenderness was observed in the standardized cooking time when the pieces were stuck with a fork.

Browning of the meat for the brown stews was done in a preheated saucepan. For preheating, the saucepan was covered with a tin lid. Through a cork in the lid, a thermometer was adjusted to just clear the bottom of the pan. The saucepan was heated over a high flame until the thermometer registered 428°F.(220°C.), then 1 teaspoon of cooking oil was added, and the pan was tilted quickly to cover the surface with fat. The meat was placed in the pan. The pan temperature during browning was controlled by maintaining a definite gas pressure with a mineral oil manometer. As the meat browned, it was stirred with a spatula. The browning period was 10 minutes.

Tap water was added to the meat. For brown stews, the water was cool because there was less spattering than when it was hot. For light stews, however, the water

was hot because when boiling water was added to the cool meat, the stew reached cooking temperature more quickly. The volume of broth desired was about 100 to 200 ml. The quantity of added water was varied according to the cooking temperature and whether or not the meat was browned. Suitable quantities were determined by numerous previous trials and are given in Table 1.

TABLE 1

Cooking time and volume of liquid for stewed pork

	Cooking time	Water used for cooking	Average volume of broth after cooking
	minutes	*ml.*	*ml.*
Browned			
Pressured	9	150	69
Boiled	45	300	134
Simmered	70	150	120
Not browned			
Pressured	11	75	146
Boiled	60	150	134
Simmered	110	100	208

Pressured stews were cooked at 15 pounds pressure, 250°F.(121.1°C.). At the end of the cooking period (Table 1) the saucepan was placed immediately in a dishpan of cool water and the water splashed over the top to cool the saucepan quickly (approximately 15 seconds).

For the boiled stews the lid of the saucepan was adjusted loosely after the water was added. The burner and saucepan containing the meat and water were placed on a large solution balance, and the weight at the beginning of boiling was recorded. Experience had shown that the final weight must be somewhat less than at the start to allow for the volume of the juices which leave the meat during cooking. Weighing was done frequently during cooking and measured amounts of boiling water were added when necessary to maintain the approximate water level and estimated final weight.

For the simmered stews the saucepan was covered with a tin lid having a small hole in the top through which was suspended a thermometer adjusted to the right height by a string. The temperature of the liquid was kept between 194 to 203°F.(90 to 95°C.). The thermometer was under constant observation by a worker who recorded maximum and minimum temperatures at 5-minute intervals.

Sampling for analysis. When the stews were cooked, the meat was lifted out with a silver spoon, placed in a tared covered container, weighed, and cooled in a dishpan of cool water.

The broth was poured into a graduated cylinder. The saucepan was rinsed with approximately 100 ml. of boiling water and the rinsings saved in an 800-ml. beaker. After cooling the volume of broth was recorded, the broth added to the beaker, and the cylinder rinsed with hot water from a wash bottle to remove all the protein material. Then 4.13 g. sodium acetate, 0.5 g. papain, and 0.5 g. takadiastase were added and stirred until dissolved. Acetic acid was added to pH 4.0 and the electrodes washed with hot water from a wash bottle. Then 20 drops of HCl were added, and the beakers were placed in an oven regulated at 50°C. The next morning, the beakers were placed in an ice water bath to harden the fat. The contents were stirred meanwhile. Then the solids were allowed to settle and the clear liquid decanted into Buchner funnels and filtered with suction into 1,000-ml. filter flasks. The fat and solids remaining in the beakers were washed and stirred with ice water and transferred to the funnels. The filtrate was made up to 1,000 ml. From this point, the broth was treated like the filtered meat digest.

Each sample of raw meat and the cooled cooked meat was ground through the fine plate of an electric meat grinder, mixed thoroughly, and sampled for analysis. The pH was taken on the remainder.

Chemical analyses. Analyses were made for thiamine, pantothenic acid, moisture, and ether extract on each sample.

Thiamine was determined by the thiochrome procedure described in Methods of Vitamin Assay *(1)*, modified slightly. Pantothenic acid was determined by the procedure described by Strong, Feeney, and Earle *(13)* as modified by Neal and Strong *(10)*.

Moisture and fat were determined on the same sample. Approximately 2-g. replicates of each sample were weighed by difference and wrapped in dry ether-extracted filter papers from a tared moisture can. They were dried in a vacuum oven at 95°C. for 48 hours, cooled in a desiccator and weighed. The dry paper-wrapped samples then were placed directly into the extraction chamber of a soxhlet extraction apparatus and extracted with ether for 16 hours. (Several of the meat samples could be placed in the same extractor.) After extraction the paper-wrapped samples were removed and placed on a clean towel to allow the ether to evaporate. They were then dried in the vacuum oven overnight and weighed.

RESULTS

The average values for the weight of meat, loss in weight during cooking, moisture, ether extract, dry fat-free solids, and pH are given in Table 2. Loss of weight during cooking was around 40%. The moisture content of the meat decreased markedly during cooking. The percentage of dry fat-free solids was much larger in the cooked meat than in the raw. Browning increased the dry fat-free solids slightly but the difference was highly significant statistically.

TABLE 2

Various information obtained on the pork stews

Description of meat	Weight of meat	Loss in weight during cooking	pH of meat	Moisture	Ether extract	Dry fat-free solids
	g.	*%*		*%*	*%*	*%*
Raw	500	....	5.8	68.9	12.0	19.1
Browned						
Pressured	283	43	6.1	54.9	14.2	30.9
Boiled	288	42	6.1	55.0	14.6	30.4
Simmered	298	40	6.1	54.2	15.9	29.9
Not browned						
Pressured	293	41	6.1	56.4	14.0	29.7
Boiled	306	39	6.1	56.4	15.3	28.4
Simmered	314	37	6.1	56.1	16.3	27.6

The pH of the raw pork averaged 5.8 with a range of 5.7 to 5.9 while that of the cooked pork averaged 6.1 with a range of 6.0 to 6.2. The pH of cooked pork did not vary with the method of cooking but was always higher than that of the raw pork.

The average thiamine and pantothenic acid content of raw and cooked meat (on the moist and dry fat-free basis) and of the broth (on the moist basis) are given in Table 3. The total thiamine content of the broth was affected rather markedly by browning. The pantothenic acid content also was lower in the broth of the browned stews. The thiamine values for the raw pork in this study ranged from 2.92-3.54 γ per g. with an average of 3.28 γ per g. These values are rather low for pork inasmuch as values of 10 γ per g. or over have been reported by Cover, Dilsaver, and Hays *(4)*, Cover, Dilsaver, Hays, and Smith *(5)*, Hewston and Marsh *(8)*, and McIntire, Schweigert, Henderson, and Elvehjem *(9)*.

Retentions were calculated on the moist basis by using total content of the raw stew as 100% and the combined total content from the cooked

meat and broth as the value for the cooked stew. This was done because in recipes using stewed pork the small amount of broth is always used. The average retentions calculated in this way are given in Table 3.

Pantothenic acid retentions in these pork stews averaged: for browned stews as pressured, boiled, and simmered, 79%, 75%, and 83%, respectively; for light stews 88%, 95%, and 97%, respectively. The average for all of the brown stews was 79% and for all of the light stews was 93%. This difference is highly significant statistically. However, the retentions did not differ significantly among the temperatures of cooking (pressured, boiled, and simmered).

TABLE 3

Thiamine and pantothenic acid content and retention of pork stews

Description of meat	Content						Retention[a]	
	Meat				Broth			
	Moist basis		Dry fat free basis		Total content of broth		Thiamine	Pantothenic acid
	Thiamine	Pantothenic acid	Thiamine	Pantothenic acid	Thiamine	Pantothenic acid		
	γ per g.	γ per g.	γ per g.	γ per g.	γ	γ	%	%
Raw	3.28	7.47	17.23	39.02	.....	.....	.....	.....
Browned								
Pressured	2.25	6.87	7.29	22.19	203	975	51	79
Boiled	1.84	5.45	6.07	17.83	283	1194	50	75
Simmered	2.30	6.59	7.69	21.93	273	1128	58	83
Not browned								
Pressured	2.23	6.26	7.53	21.05	537	1418	72	88
Boiled	2.08	6.26	7.33	22.01	561	1561	73	95
Simmered	2.10	5.83	7.62	21.02	599	1726	76	97

[a] Retentions were calculated on the moist basis by using total content of the raw stew as 100% and the combined total from the cooked meat and broth as the value for the cooked stew.

Thiamine retentions in pork stews prepared in the 6 different ways averaged: for browned stews cooked by pressuring, by boiling, and by simmering 51%, 50%, and 58%; and for light stews, 72%, 73%, and 76%, respectively. The average for all of the brown stews was 53% and for all of the light stews 74%. This difference is highly significant statistically. However, retention did not differ significantly among the 3 temperatures of cooking (pressured, boiled, and simmered). Cover, Dilsaver, and Hayes *(3)* reported that the retentions of thiamine in beef and lamb stews were affected significantly by browning but not by the different cooking temperatures. Thus the results for pork stews fall in line with those for beef and lamb stews in regard to the statistical significance of the effect produced by browning and by the 3 cooking temperatures.

One way of assessing the nutritive effect of a cookery process on a food is to relate the amount of the nutrient present in a serving of the food to the recommended daily allowance. If we assume a serving of pork to be 100 g. on the raw moist basis, then a serving of pork stew would average 0.174 mg. of thiamine if the stew had been browned and 0.243 mg. if it had not been browned. Using 1.5 mg. as the recommended daily allowance (6), these browned pork stews furnished 12% and the light pork stews furnished 16% of the thiamine recommended for 1 day. It is doubtful

whether this small difference in thiamine caused by browning is significant in the practical nutrition of well nourished people. However, if the thiamine content of this pork had been as high as 10 γ per g. (as has been reported many times for fresh pork), then the brown stews could have furnished about 35% and the light stews about 50% of the daily allowance. Thus the saving in thiamine by not browning the meat would have amounted to 15% or about $\frac{1}{7}$ of the entire amount recommended for one day and could be important in practical nutrition. It has been shown by Pyke *(12)*, Pence, Miller, Dutcher, and Ziegler *(11)*, and Hartzler, Ross and Willet *(7)* that the amount of thiamine in the feed of the pigs is reflected in the thiamine content of their meat. Thus the higher the thiamine content of the feed, the greater could be the difference between browning and not browning during cooking so far as practical nutrition is concerned.

SUMMARY

In a study of the retention of the B vitamins in meat stews, the effect of different cooking procedures on the retention of thiamine and pantothenic acid was determined when pork stews were browned or not browned, simmered or boiled, or cooked at 15 p.s.i.g. pressure. Browning caused a significant loss of both vitamins, but the retention of thiamine and of pantothenic acid did not differ significantly when the various cooking temperatures were used.

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THERMAL DESTRUCTION OF CAMERON'S PUTREFACTIVE ANAEROBE 3679 IN FOOD SUBSTRATES[a]

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Cameron's Putrefactive Anaerobe 3679 is widely used as a test organism to evaluate process requirements for low-acid canned foods. Although the thermal resistance of this organism is known to vary with the substrate in which it is heated *(2, 15, 18)*, until recently *(10, 16)* there were relatively few data in the literature that defined the constants of thermal-death-time curves of the organism in vegetable substrates. Most of these data specify the resistance of spores when heated in liquid extracts or puréed vegetables. Townsend, Esty, and Baselt *(18)* have emphasized the need for information based on work with raw products rather than with brines from processed foods, and data reported more recently by Reed, Bohrer, and Cameron *(10)* indicate that heat resistance of spores in a given vegetable substrate may differ considerably, depending on whether the substrate is prepared as a purée or as larger pieces suspended in brine. With respect to processes for nonpuréed vegetables, it seems reasonable to assume that the latter medium would be superior to the purée in providing environmental conditions that simulate conditions to which spores are exposed in containers of vegetables during processing.

The study reported in this article, was undertaken to augment currently available data by determining the constants of thermal-death-time curves of P. A. 3679 spores heated in 13 low- and medium-acid vegetables, chopped or cut.

Terms and nomenclature used in this paper are consistent with those used by Ball *(3)*, Townsend, Esty, and Baselt *(18)*, Sognefest and Benjamin *(14)*, Reed, Bohrer, and Cameron *(10)*, and others.

EXPERIMENTAL METHODS

Preparation of spore suspensions. Spore suspensions of P. A. 3679 were prepared by culturing the organism in 10% egg-meat medium (Difco) at 30°C.(86°F.) for 3 weeks, filtering the medium through glass wool, centrifuging the filtrate, and suspending the spores in a small volume of sterile 0.5% peptone solution. Concentrated suspensions, containing 3×10^8 to 5×10^8 spores per ml., were distributed in 2 to 4 ml. quantities in TDT (thermal-death-time) tubes. The tubes were sealed and heated for 20 minutes at 100°C.(212°F.) *(12)* and held under refrigeration until used.

Preparation of substrates. Both commercially frozen and fresh food samples were used for preparing food substrates. Frozen foods were thawed; fresh vegetables were washed, trimmed, and blanched. All samples, with the exception of asparagus and spinach, were chopped in a hand food chopper[b]. Asparagus and spinach were chopped

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[b] Cat. No. 8-490, Fisher Scientific Co., New York, N. Y.

with a knife to pieces ⅛ inch or less. Fill-in weights of food substrate and liquid were determined by the amount of substrate necessary to pack TDT cans to a depth of 3/16 of an inch and the amount of water or spore suspension just sufficient to cover the food. Sørenson's phosphate buffer, pH 7.0, 0.066*M.*, was used as the reference substrate.

The methods of handling and fill-in weights for TDT cans for each of the 13 food substrates are outlined in Table 1.

TABLE 1

Preparation and fill-in weights of vegetable substrates for thermal-death-time cans

Product	Preparation	Weight of prepared vegetable per can	Volume of water per can
		g.	*ml.*
Asparagus (frozen)	Chopped	14	4
Beans, lima (frozen)	Chopped	10	5
Beans, snap	Trimmed, cut, blanched 5 minutes in boiling water, chopped	9	6
Beets	Washed, blanched 10 minutes in steam, peeled, chopped	9	4
Carrots	Washed, scraped, chopped, blanched 5 minutes in steam	9	6
Corn (frozen)	Chopped	13	2
Mushrooms (frozen)	Chopped	9	6
Okra	Trimmed, blanched 3 minutes in boiling water, chopped	9	6
Peas (frozen)	Chopped	9	6
Potatoes, sweet	Peeled, chopped, blanched 1 minute in steam	9	6
Pumpkin	Peeled, chopped, blanched 1.5 minutes in steam	9	6
Spinach	Washed, trimmed, blanched 10 minutes in steam, chopped	11	3
Squash, summer	Washed, chopped, blanched 1 minute in steam	9	6

Thermal-death-time studies. Thermal-death-time data for P. A. 3679 spores heated in food substrates were obtained by processing vegetable packs in 208 × 006, enameled TDT cans *(18)*. The number of cans processed at each time interval ranged from 3 to 24. Range finding runs were made with 3 to 4 cans per interval and final end points checked with 12 or more cans per interval. The inoculum of 10,000 spores per can was added in the water used to cover the chopped vegetables after packing, and was distributed evenly over the pack. Cans were sealed under a vacuum of 19 inches before processing.

With the exception of lima beans, corn, and peas, 2 methods were used for establishing the end points of thermal-death-time determinations. For a measure of the heat treatments required to obtain practical or commercial sterility with respect to P. A. 3679 spores, processed vegetable packs were incubated in the cans without further treatment. With all determinations in vegetables in which product inhibition of spore germination was considered possible, parallel series were processed and incubated under conditions more favorable for development of the test organism. For these tests an in-can incubation technique, in which a measured amount of buffered concentrated peptone medium was added to each can before incubation, was used. After processing, a small hole was punched in the side of the can and 1.5 ml. of sterile medium added by means of a syringe. The hole was sealed immediately with a drop of solder. Aseptic techniques were used throughout.

The medium used in the above tests consisted of 7.5% tryptone (Difco); 2.5% yeast extract (Difco); 0.5% sodium thioglycollate (BBL); 2.72% KH_2PO_4; and 0.74% NaOH. Repeated determinations made after the addition of the medium to the food

substrates indicated a final pH for the can contents ranging from pH 6.6 to pH 7.2.

Cans were incubated at 30°C.(86°F.) for a minimum period of 15 weeks. Germination of the spores and proliferation of the organism were indicated by swelling of the cans. Contents of some of the swollen cans were also checked by microscopic examination for confirmatory evidence of spore germination and growth.

Thermal-death-time cans were heated in a miniature retort constructed from a pressure saucepan (Figure 1). The retort was equipped with cold water, steam, vacuum, and drain lines at the bottom, an overflow line in the upper body, and a bleeder valve, pressure-vacuum gauge, and a stuffing box in the cover. All lines were equipped with manually operated valves. The cans to be heated were placed on their sides in a wire basket with divisions to space the cans, and the basket supported ½ inch from the bottom of the retort. A free flow of steam around each can was thus assured during processing.

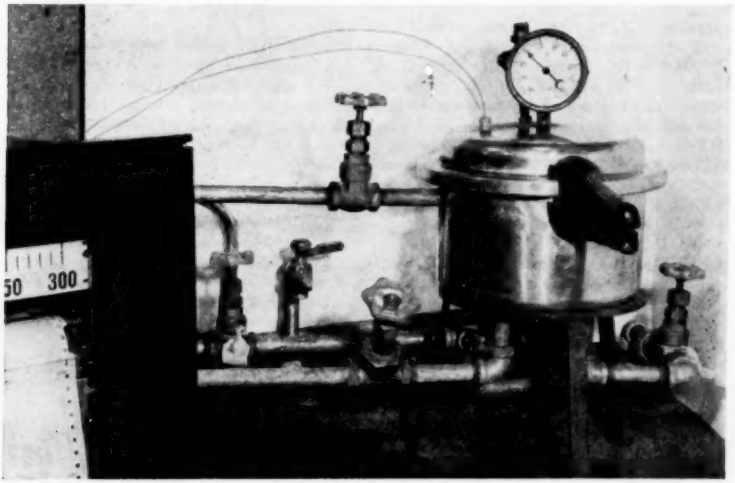

Figure 1. Experimental retort used for heat penetration and thermal-death-time determinations.

In making heat penetration and thermal-death-time determinations, the retort was loaded and closed, cold water run through the retort for 3 or 4 minutes to bring the cans to the same initial temperature, the water drained, and a vacuum of 18-20 inches drawn on the retort. The vacuum line was then closed, the steam line opened, and timing of the process begun. At the conclusion of the process the steam supply was cut and the cold water and overflow lines opened. Come-up time for the retort to 250°F. (121.1°C.) as observed for 99 determinations varied between 13 and 20 seconds with a mean of 16.5 seconds. These results compare closely with the come-up time of 15 to 20 seconds reported by Sognefest and Benjamin *(14)*.

Reference curves for P. A. 3679 spores, heated in neutral phosphate buffer, were based on both thermal-death-time and rate determinations. Thermal-death-time determinations were carried out at 239°F.(115°C.) at frequent intervals during the course of the work to provide a check on the resistance, and stability during storage, of spore suspensions used. These determinations were made by heating 2-ml. aliquots of suspensions sealed in Pyrex TDT tubes following the method of Bigelow and Esty *(5)* with the following modifications: Three TDT tubes were used at each time interval and subculturing was carried out in tryptone yeast extract agar using flat oval culture tubes (9). The inoculated agar was solidified quickly by immersing tubes in ice water

and was then layered with approximately 1 inch of sterile 1.5% agar. Recovery tubes were incubated at 30°C.(86°F.) for a minimum period of 3 weeks. Spore concentration was 100,000 per ml. Heating was carried out in an oil bath equipped with a stirrer and thermostatically controlled to ±0.3°C.(0.5°F.).

The slope of the phosphate reference curve was based on death rate determinations carried out at 3 different temperatures. These determinations were made in essentially the same manner as the thermal-death-time determinations except that tubes were removed at shorter intervals from the oil bath, and viable counts, in tryptone yeast extract agar, were made on appropriate dilutions of the heated suspensions. The resulting data were plotted to give survivor curves from which time intervals corresponding to 99.99% destruction were determined. These points served for the construction of a "phantom" thermal-death-time curve, the position of the curve being established by the survival-destruction intervals determined at 239°F.(115°C.).

Recovery medium used in the above rate and time determinations had the following composition: 1.5% tryptone (Difco); 0.5% yeast extract (Difco); 0.1% sodium thioglycollate (BBL); 1.5% agar (Difco); pH adjusted to 7.4.

Heat penetration. In order to determine the correction factor for the lethal value of the lag time occurring during heating, heat penetration data were obtained with buffer in TDT tubes and food substrates in TDT cans. For the TDT tubes, copper-constantan[c] thermocouples were sealed into thin glass supporting tubing with a cement that expands on setting[d] and the glass assemblies then ring-sealed inside the TDT tubes. The thermocouple junction was placed $\frac{5}{8}$ inch from the bottom of the TDT tubes containing 2 ml. of liquid. For the TDT cans, a short length of concentric copper-copnic thermocouple wire[e] was led into the cans through a hole in the side of the can and positioned with rubber spacers *(14)*. Copper-copnic thermocouples have the same temperature-millivolt relationship as copper-constantan thermocouples. The wire was then sealed into the hole with solder, the can filled with food substrate and water and sealed, and the protruding wire soldered to a more rigid copper-constantan lead. The concentric thermocouple wire facilitated the formation of an airtight seal where the wire entered the can without the use of a packing joint. Temperature readings were recorded with a recording potentiometer[f]. Tubes were heated in an oil bath and cans in the steam retort described earlier. Correction factors were calculated for a z value of 18 from the slowest heating curves, using the mathematical method of Ball *(3)*.

RESULTS

Heating lag correction factors. When TDT tubes were preheated in boiling water for 15 seconds before transfer to the oil bath, the slowest heating curve was characterized by f_h and j values of 1.50 and 0.52, respectively. Without the preheat, the corresponding constants were 1.57 and 0.62. The calculated lag correction factors, of 1.35 minutes with preheating and 1.71 minutes without, are presented with the heat penetration data in Table 2. Characteristics of heating curves and calculated correction factors for 13 vegetables packed in TDT cans and heated in steam at 250°F. (121.1°C.) are summarized in the same table. The f_h values derived from the slowest heating curves observed for each of the vegetable packs varied from 0.85 to 1.13 minutes and j values from 1.14 to 1.93. Calculated minimum and maximum correction factors were 1.41 and 1.81 minutes, respectively, with a mean of 1.67 minutes. All TDT determinations were corrected by the factor for the corresponding product as listed in Table 2.

[c] No. 24 B and S gauge, duplex, enamel-glass insulation.

[d] X-Pandotite, X-Pando Corporation, Long Island City, N. Y.

[e] General Electric sheath type 0.009 inch copnic wire with "Formex" insulation inside a 0.018 inch O. D., 0.003 inch wall copper tube.

[f] Brown Electronik Strip Chart Pyrometer, Multiple Point Recorder, High Speed. Brown Instrument Co., Philadelphia, Pa.

Sognefest and Benjamin *(14)* have noted that the correction factor does not vary significantly with processing temperatures over a range of 230°F. (110°C.) to 270°F.(132.2°C.). Accordingly the correction factors calculated here for 250°F.(121.1°C.) were applied in correcting heating times at other temperatures used in this work.

TABLE 2

Characteristics of slowest heating curves and lag correction factors for various substrates experimentally heated in thermal-death-time tubes and cans [a]

Product	Container	Retort temp.	Initial temp.	No. runs	f_h	j	Correction factor [b]
		°F.	°F.		*minutes*		*minutes*
Buffer [1]	Tube	240	175	8	1.57	0.62	1.35
Buffer	Tube	240	86	2	1.50	0.52	1.71
Asparagus	Can	250	59	8	.92	1.62	1.63
Beans, lima	Can	250	59	9	.93	1.83	1.65
Beans, snap	Can	250	58	6	.95	1.82	1.71
Beets	Can	250	57	8	1.13	1.14	1.81
Carrots	Can	250	57	7	.85	1.29	1.41
Corn	Can	250	61	7	1.03	1.43	1.75
Mushrooms	Can	250	59	12	1.03	1.52	1.78
Okra	Can	250	59	8	.90	1.62	1.60
Peas	Can	250	58	6	.97	1.93	1.78
Potatoes, sweet	Can	250	56	8	.93	1.65	1.63
Pumpkin	Can	250	59	6	.92	1.57	1.60
Spinach	Can	250	54	8	.88	1.79	1.59
Squash, summer	Can	250	58	7	1.00	1.67	1.77

[a] TDT tubes heated in oil; TDT cans, in steam.
[b] Correction factors based on a z value of 18°F. and a heating time greater than B" minutes: the time necessary to obtain a temperature 0.1°F. below retort temperature *(3)*.
[1] Preheated in boiling water for 15 seconds.

Thermal-death-time curves. End points of thermal-death-time determinations for P. A. 3679 spores heated in the various food substrates are presented in Table 3, and F and z values of the thermal-death-time curves constructed from the data in Table 4. Observed z values ranged from 14.5 to 27.5 when the curves were based on food substrate incubation results. When based on data obtained by incubation of food substrates to which medium had been added, the z values ranged from 13.5 to 23. Omitting the curve for mushrooms with a z of 13.5, the remaining values ranged from 20 to 23. F values ranged from 1.7 to 10.5 for curves based on food substrate incubation alone, while the range for food substrates supplemented with medium varied between 4.7 and 9.6. In all instances where determinations were made by both methods, highest F values were obtained with food substrates incubated after supplementation with the peptone medium.

To further evaluate the data at hand, "phantom" thermal-death-time curves *(4)* were constructed from D values calculated by the methods described by Stumbo *(16)* and by Schmidt *(13)*. Application of the latter method, based on Reed's *(11)* technique for treating bioassay data, is illustrated in Table 5. The figures in column "m" represent the cumulative total of cans showing surviving spores, starting with the longest

TABLE 3

Survival and destruction points for 10,000 P. A. 3679 spores heated in vegetable substrates in thermal-death-time cans with and without medium supplementation prior to incubation

Product	Media	Temperature	Survival	Destruction
		°F.	*minutes*	*minutes*
Asparagus	+	250	5.5	6.4
		245	9.7	11.7
		240	16.7	19.2
		235	28.7	34.2
	−	250	2.2	3.7
		245	6.7	9.2
		240	12.7	15.7
		235	.....	18.2
Beans, lima	+	.....	.....	.....
	−	250	9.7	10.7
		245	20.7	23.2
		240	49.8	58.7
Beans, snap	+	250	6.3	7.4
		245	9.8	11.1
		240	14.6	17.1
		235	28.6	32.6
	−	250	1.1	3.0
		245	3.6	4.6
		240	7.6	8.6
		235	11.6	13.1
Beets	+	250	6.5	7.4
		245	11.5	15.0
		240	19.0	22.5
		235	35.0	41.5
	−	250	2.0	3.0
		245	5.5	6.5
		240	8.0	10.0
		235	16.0	18.5
Carrots	+	250	4.9	6.4
		245	8.4	12.4
		240	18.0	20.0
		235	29.9	33.9
	−	250	1.4	2.4
		245	.....	2.4
		240	5.9	6.9
		235	12.9	15.4
		230	23.9	27.9
Corn	+	.....	.....	.....
	−	250	8.7	10.2
		239	37.7	44.7
		231.8	86.7	98.7
		224.6	148.7	173.7
Mushrooms	+	250	4.5	6.0
		245	12.5	16.5
		240	28.5	34.5
	−	250	4.5	6.0
		245	12.5	16.5
		240	26.5	28.5

TABLE 3 (Concluded)

Survival and destruction points for 10,000 P. A. 3679 spores heated in vegetable substrates in thermal-death-time cans with and without medium supplementation prior to incubation

Product	Media	Temperature	Survival	Destruction
		°F.	*minutes*	*minutes*
Okra	+	250	3.2	4.1
		245	7.9	8.7
		240	13.7	14.7
	−	250	0.7	2.2
		245	4.4	5.4
		240	8.7	11.2
		235	.....	13.7
Peas	+	.....	.....	.....
	−	250	5.5	7.0
		245	11.5	12.2
		240	28.5	33.5
Pumpkin	+	250	3.7	5.7
		245	8.7	11.7
		240	15.2	18.7
		235	30.7	33.7
	−	250	1.2	2.2
		245	3.7	5.2
		240	5.7	7.7
		235	12.2	13.7
Potatoes, sweet	+	250	7.2	8.7
		245	10.7	12.2
		240	20.7	22.7
		235	41.4	46.4
	−	250	4.2	6.2
		245	8.7	10.7
		240	11.2	13.7
		235	19.7	21.7
Spinach	+	250	8.7	10.2
		245	8.7	13.7
		240	19.7	25.7
		235	48.7	52.7
	−	250	4.7	5.5
		245	8.7	11.2
		240	15.7	20.7
		235	32.7	36.7
Squash, summer	+	250	7.3	8.5
		245	12.5	15.0
		240	23.5	26.7
		235	33.5	43.5
	−	250	2.5	4.0
		245	2.7	5.5
		240	11.5	12.7
		235	20.0	22.5

heating time, while the figures in column "n" represent the cumulative total of cans showing no survivors, starting with the shortest heating time. The probability of sterility at each time interval is presented in column "*P*" where "*P*" equals $(n + 1)/(m + n + 2)$. The probability data so obtained are plotted against time on arithmetic probability paper. The time corresponding to LD_{50}, the point on the curve where the probability that a can is either sterile or contains viable organisms is equal (*P* is 0.5), is noted and *D* values, presented in the last column, are calculated from the formula *D* equals time at $(LD_{50})/(\log \text{initial number of spores per tube} - \log 0.5)$.

TABLE 4

Characteristics of thermal-death-time curves constructed from survival and destruction points for P. A. 3679 spores heated in neutral phosphate buffer and in 13 vegetable substrates

Substrates	*z*		*F*	
	Without medium	With added medium [j]	Without medium	With added medium [j]
	minutes	*minutes*	*minutes*	*minutes*
Buffer[k]	.....	21 [l]	.....	8.9 max. 5.5 min.
Asparagus	16	21	3.5	6.0
Beans, lima	14.5	.....	10.5	.....
Beans, snap	20.5	23	2.5	6.7
Beets	21.5	20	3.3	6.8
Carrots	16	20	1.7	6.0
Corn	18.5	.....	10.0	.....
Mushrooms	14.5	13.5	5.8	5.8
Okra	14.5	21.5	2.1	4.7
Peas	14.5	.....	6.0	.....
Potatoes, sweet	27.5	20	5.8	7.6
Pumpkin	19	20	2.2	6.0
Spinach	18	20.5	5.0	9.6
Squash, summer	20	22	3.8	8.2

[j] Medium added to thermal-death-time cans after processing and prior to incubation.
[k] Subcultured from thermal-death-time tubes.
[l] From thermal-death-rate determinations.

A few *D* values were calculated by the most probable number method of Halvorson and Ziegler *(8)* as described by Stumbo, Murphy, and Cochran *(17)*. This method, however, was not routinely used since no practical differences were observed between thermal-death-time curves based on these values and those obtained with Stumbo's earlier method *(16)*. In conformance with recent practice *(10, 17)*, the symbol *D* was used herein, replacing the symbol *Z* (zeta) *(4)*, to express the heating time necessary for the destruction of 90% of viable spores. The *z* values for the curves established by both methods were generally in close agreement for any particular food substrate where data were available for comparison. Both methods, in turn, produced "phantom" thermal-death-time curves whose *z* values were generally in close agreement with *z* values obtained from the thermal-death-time curves based on survival-destruction points.

In several instances thermal-death-time curves could not be constructed by Stumbo's method because of an insufficient number of points (*D* values) to plot the curve. This method utilizes data at any specific time interval only when there are both survivals and deaths during the heating period. *D* values calculated by the methods of Stumbo *(16)* and Schmidt *(13)* are presented in Table 6, and Table 7 presents the characteristics of "phantom" thermal-death-time curves constructed from these values.

TABLE 5

Calculated time by Schmidt's method for 90% destruction of 10,000 P. A. 3679 spores heated at 250°F.(121.1°C.) in carrots [m]

Heating time	Number cans heated	Number survivals	Number deaths	m	n	m + n	P [a]	LD_{50}	D [o]
minutes									
1.4	12	12	0	52	0	52	0.019		
1.9	12	9	3	40	3	43	0.089		
2.8	12	11	1	31	4	35	0.135		
3.3	13	9	4	20	8	28	0.300		
4.0	12	1	11	11	19	30	0.625		
4.9	12	10	2	10	21	31	0.667		
6.4	4	0	4	0	25	25	0.963		
7.4	12	0	12	0	37	37	0.974		
								3.75	0.872

[m] Incubated with medium.

[a] See text for formula used in calculating *P*.

[o] See text for formula used to calculate *D*.

From thermal-death-rate determinations, times required for 99.99% destruction of P. A. 3679 spores when heated in neutral phosphate buffer were 15.3 minutes at 239°F.(115°C.); 37.2 minutes at 231.8°F.(111°C.); and 76.3 minutes at 224.6°F.(107°C.). These points represent averages of 3 determinations each at 224.6°(107°C.) and 239°F.(115°C.) and 2 at 231.8°F.(111°C.). When plotted (Figure 2), the resulting "phantom" thermal-death-time curve has a *z* value of 21. The *F* value based on 99.99% destruction equals 4.8 minutes. Among 20 thermal-death-time determinations at 239°F.(115°C.), which were carried out on P. A. 3679 spore suspensions over a period from April 1948 to February 1951, survival-destruction intervals ranged from a low of 17.5 minutes survival and 19.5 minutes destruction to a high of 27.5 minutes survival and 31.5 minutes destruction. Mean survival-destruction interval was 23.5 minutes survival and 26.5 minutes destruction. Thermal-death-time curves drawn parallel to the "phantom" curve and passing through the minimum, average, and maximum observed intervals are also illustrated in Figure 2. These curves indicate a probable maximum phosphate buffer *F* value of 8.9, a minimum *F* value of 5.5, and an average *F* value of 7.5 minutes for the 100,000 per ml. concentration of the P. A. 3679 spore suspensions used in this work. Because of the limited number of tubes used at each interval in making the determinations, too much significance cannot be given to the lower-resistance values observed *(7)*. Had more tubes been used, *F* values equal to or approaching 8.9 would presumably have been obtained.

TABLE 6

Calculated times for 90% destruction of 10,000 P. A. 3679 spores heated in 13 vegetables and incubated with and without added medium

Product	Medium	*D* values for P. A. 3679 spores at											
		250°F.		245°F.		240°F.		235°F.		230°F.		224.6°F.	
		A[b]	B[c]	A	B	A	B	A	B	A	B	A	B
		minutes	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*	*minutes*
Asparagus	+	1.10	1.14	2.17	2.12	3.93	3.95	6.34	7.09	.....	.....	.....	.....
	−	0.50	0.47	1.56	1.56	2.50	2.61	.....	.....	.....	.....	.....	.....
Beans, lima	+	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	−	1.74	1.49	4.33	4.19	9.82	10.81	.....	.....	.....	.....	.....	.....
Beans, snap	+	1.07	1.05	2.08	2.02	3.40	3.40	6.65	6.65	.....	.....	.....	.....
	−	0.26	0.26	0.74	0.65	1.43	1.49	.....	2.91	.....	.....	.....	.....
Beets	+	1.18	1.28	2.49	2.56	.....	4.81	7.61	7.74	.....	.....	.....	.....
	−	0.48	0.53	1.20	0.97	1.86	1.86	3.31	3.26	.....	.....	.....	.....
Carrots	+	0.79	0.87	1.68	.....	4.01	4.13	6.50	5.19	.....	.....	.....	.....
	−	0.34	0.37	.....	.....	1.21	1.22	2.40	2.09	4.60	4.63	.....	.....
Corn	+	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	−	2.11	2.14	.....	.....	9.20[f]	9.33[f]	.....	.....	.....	19.40[g]	.....	37.44
Mushrooms	+	0.61	1.16	.....	3.37	5.82	6.86	.....	.....	.....	.....	.....	.....
	−	.....	1.22	2.91	2.91	5.24	4.98	.....	.....	.....	.....	.....	.....
Okra	+	0.69	0.65	1.65	1.67	2.52	2.56	.....	.....	.....	.....	.....	.....
	−	.....	0.34	0.85	0.86	1.94	1.86	.....	.....	.....	.....	.....	.....
Peas	+	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....
	−	1.20	1.12	2.50	2.63	5.94	4.77	.....	.....	.....	.....	.....	.....
Potatoes, sweet	+	1.22	1.00	2.34	2.33	4.11	3.61	.....	10.26	.....	.....	.....	.....
	−	0.85	0.84	1.28	1.09	2.29	2.44	.....	4.77	.....	.....	.....	.....
Pumpkin	+	.....	1.09	1.89	1.93	3.31	3.42	6.71	6.65	.....	.....	.....	.....
	−	.....	0.40	0.86	0.76	.....	1.57	2.59	2.97	.....	.....	.....	.....
Spinach	+	1.73	1.69	2.08	2.63	4.69	4.83	11.32	11.33	.....	.....	.....	.....
	−	1.00	1.09	1.94	1.81	3.41	3.02	6.67	6.86	.....	.....	.....	.....
Squash, summer	+	1.32	1.42	2.32	.....	5.29	5.47	7.28	6.28	.....	.....	.....	.....
	−	0.60	0.66	.....	.....	2.57	2.33	3.80	3.03	.....	.....	.....	.....

[b] Stumbo's method *(16)*. [c] Schmidt's method *(13)*. [f] *D* value at 239°F. [g] *D* value at 231.8°F.

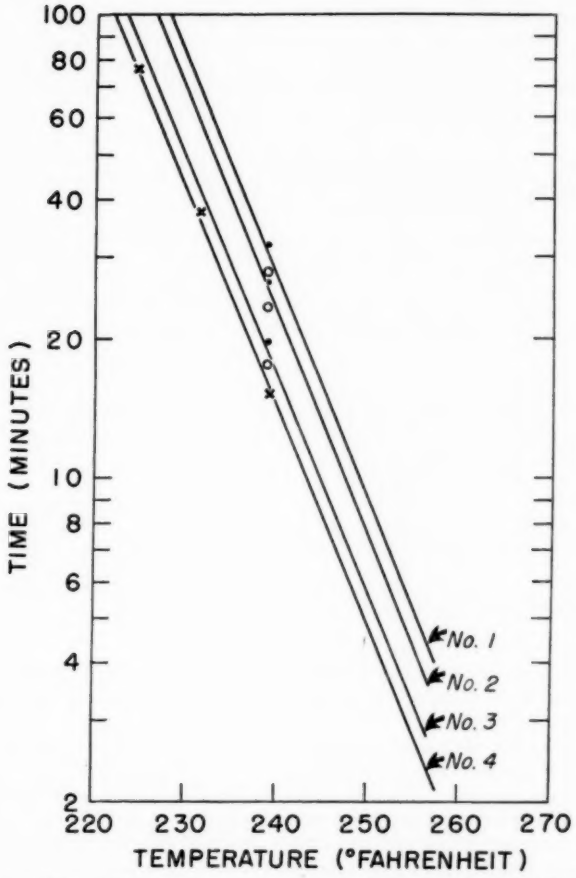

Figure 2. Thermal-death-time curves for P. A. 3679 in neutral phosphate buffer. Curve No. 1: Based on maximum survival and destruction points at 239°F.(115°C.); curve No. 2: Based on average survival and destruction points at 239°F.(115°C.); curve No. 3: Based on minimum survival and destruction points at 239°F.(115°C.), and curve No. 4: Based on 99.99% destruction of spores.

Spores of P. A. 3679 produced in the egg-meat media have been of uniformly high resistance, and spore yields have been greater than in other media tested. Typical yields, as viable spores per ml. of media, are shown in Table 8. The counts, made on heat-activated suspensions in tryptone-yeast extract agar with and without added bicarbonate (0.2 ml. sterile 10% sodium bicarbonate to 15 ml. medium), confirm Andersen's *(1)* findings of the stimulatory effect of bicarbonate on germination of anaerobic spores.

TABLE 7

z values of "phantom" thermal-death-time curves for P. A. 3679 spores heated in 13 vegetables and incubated with and without medium supplementation

Substrate	*z* values			
	Stumbo's method		Schmidt's method	
	Without medium	With medium	Without medium	With medium
	minutes	*minutes*	*minutes*	*minutes*
Asparagus	.....[t]	20	.....[t]	19
Beans, lima	13.5	.....	12	.....
Beans, snap	.....[t]	18.5	14	18.5
Beets	19.5	19	19	19
Carrots	17.5	15.5	18.5	.....[t]
Corn	.....[t]	.....	19.5	.....
Mushrooms	.....[t]	.....[t]	16.5	13
Okra	.....[t]	.....[t]	14	.....[t]
Peas	14.5	.....	15.5	.....
Potatoes, sweet	24	20.5	20.5	15
Pumpkin	.....[t]	18	17	19.5
Spinach	18	17.5	18	18.5
Squash, summer	.....[t]	18.5	20	.....[t]

[t] Insufficient points for plotting of curve.

DISCUSSION

As pointed out by Reed, Bohrer, and Cameron *(10)*, calculated process times become longer as *z* values are decreased. The *z* values reported here for P. A. 3679 thermal-death-times curves range from 13.5 to 23 when based, with the exception of lima beans, corn, and peas, on end points observed following incubation in medium-enriched vegetable substrates. The average *z* value is 19.2 with only those for lima beans, mushrooms, and peas falling below 18, while in 9 products, thermal-death-time curves had *z* values of 20 or greater. With respect, therefore, to total lethal requirements, processes based on *z* equal to 18, and *F* values required for destruction of P. A. 3679 spores in unmodified vegetable substrates, should be somewhat more than adequate to control spoilage by this type of organism in most of the products studied. For the 3 products noted above, in which *z* values were less than 18, process evaluation may need to take into consideration the observed *z* values in relation to the resistance of the test organism.

It is axiomatic that processes for medium- and low-acid foods that are subject to spoilage by toxin-producing *Clostridium botulinum* must meet the minimum requirement of being adequate to destroy spores of that organism.

In evaluating the safety of processes, available data indicate that those with lethalities yielding practical sterility with respect to P. A. 3679 spores, will provide for a margin of safety beyond sterility with respect to *Cl. botulinum*. The early work of Esty and Meyer *(6)* defined the ideal thermal-death-time curve for *Cl. botulinum* spores in phosphate buffer with F and z values of 2.78 and 18, respectively. Subsequent investigations have tended to confirm these values, though corrected F and z values of 2.45 and 17.6 have been proposed as a more accurate interpretation of the original data *(18)*. Thus the maximum reported F value for *Cl. botulinum* spores in neutral phosphate buffer is considerably lower than the values observed in these studies with P. A. 3679 spores heated under similar conditions. This relative difference in heat resistance between P. A. 3679 and *Cl. botulinum* spores has also been reported in vegetable substrates *(10, 17)*. For practical purposes, therefore, it would appear safe to assume that processes calculated for $z = 18$ and providing F_0 values equivalent to the observed F values for destruction of P. A. 3679 spores in unmodified vegetable substrates will be adequate to sterilize with respect to *Cl. botulinum* spores.

TABLE 8

Yields of P. A. 3679 spores in egg-meat media cultures

Culture No.	Spore counts in tryptone yeast-extract agar	
	With bicarbonate[a]	Without bicarbonate
	Spores per ml. $\times 10^7$	*Spores per ml.* $\times 10^7$
1	3.02	1.37
2	2.19	.48
3	1.32	.46

[a] 0.2 ml. of 10% $NaHCO_3$ per 15 ml. of media.

The precision with which processes can be evaluated depends finally upon the accuracy with which the characteristics of thermal-death-time curves of spoilage organisms can be defined. Currently available data point to variations in F and z values for a given organism when heated in a single vegetable (depending on whether the vegetable is prepared as a purée or as larger particles suspended in brine), and to differences in values obtained with the same organism in different laboratories *(10, 16)*. The z values reported here (Table 4) frequently differ depending on the technique applied to their determination. The z values were usually higher and, with 1 exception, apparent F values were considerably higher when derived from data based on recoveries during incubation in medium supplemented vegetable substrates than they were when derived from data based on incubation in unsupplemented substrates. These results show that, with the products on which parallel data were obtained, all except mushrooms were inhibitory in varying degrees to germination and growth of spores of the test organism.

The observed product inhibition makes it probable that z values based on end point data derived from the supplemented vegetable media more accurately reflect relative death rates at different temperatures. It may

also be noted that these values agree in general with those reported by Reed, Bohrer, and Cameron *(10)*.

Variation in z values is also noted depending on whether the curves are constructed from survival-destruction intervals (Table 4) or from calculated D values (Table 7). These differences may well be more apparent than real, since the experimental determinations were not designed with such calculations in mind. Calculated D values (Table 6) do, however, emphasize the effect of technique variations upon apparent death rates. D values based on recovery in supplemented vegetable media are significantly lower than those derived from recoveries in the unsupplemented vegetables. It is thus apparent that D values observed in different laboratories are comparable only when methodology has been duplicated.

SUMMARY

Data obtained by processing inoculated packs of 13 vegetables in thermal-death-time cans show that the F and z values of thermal-death-time curves for P. A. 3679 spores vary with the substrate in which they are heated.

A majority of the vegetables tested exhibited some inhibitory effect on the germination of P. A. 3679 spores.

Under the experimental conditions described and over the temperature ranges employed, thermal-death-time curves constructed from survival-destruction intervals or from calculated D values appeared to be straight lines.

The slight differences in heat penetration data and calculated heating lag correction factors observed in this investigation would seem to indicate that the heat penetration characteristics for the chopped and cut food substrates heated in steam in TDT cans are quite similar over the heating period employed. The possibility of using one lag correction factor for all the food substrates, instead of individually calculated factors for each, is thereby suggested.

The modified pressure saucepan used in this study proved to be an economical and satisfactory experimental retort.

Acknowledgment

Grateful acknowledgment is made to the American Can Company for the supply of thermal-death-time cans and the use of a No. 1 Pacific S. A. Vacuum Closing Machine.

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NUTRITIVE VALUE OF MILK PROTEINS. I. STABILITY DURING STERILIZATION AND STORAGE OF EVAPORATED MILK AS DETERMINED BY THE RAT REPLETION METHOD

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Cook, Morgan, Weast, and Parker *(2)* presented data obtained by the rat growth method which suggest that the nutritive value of evaporated milk proteins is slightly reduced during the commercial processing of evaporated milk. In some cases the differences between whole fresh and sterilized evaporated milk and between evaporated milk before and after sterilization were statistically significant. In contrast, Schroeder, Iacobellis, and Smith *(8)* found no decrease in either digestibility or biological value during the production of evaporated milk. Schroeder et al. used a dog nitrogen balance method in their investigations. Whitnah *(9)* used a rat nitrogen balance method to study losses during the processing and storage of evaporated milk. He found average values of digestibility and biological values of 93.4 and 90.5 for fresh milk, 90.9 and 87.8 for experimentally produced evaporated milk, and 91.8 and 89.4 for commercial evaporated milk. Whitnah did not study his data statistically but whether significant or not the differences are very small.

It is increasingly evident, that at least where the difference in nutritive values are slight, the biological comparison of 2 or more proteins or protein mixtures is difficult. The reader is referred to the review articles of Albanese, Chow, Frost, Mitchell, Porter, and Silber *(1)* for a discussion of the differences in the amino acid requirements in mammals and of biological methods of evaluating proteins, protein hydrolysates, and amino acid mixtures. A publication of the Rutgers University Bureau of Biological Research *(6)* presents further data illustrating the problems and inconsistencies of the various biological methods of protein evaluation. Since the data of Cook, Morgan, Weast, and Parker *(2)*, Schroeder, Iacobellis, and Smith *(8)*, and Whitnah *(9)* were not in agreement it seemed that data obtained by the rat repletion method might be of interest. The latter method was used for the studies that will be presented in this paper.

There appears to be better agreement concerning the loss of protein nutritive value during the storage of evaporated milk. Whitnah *(9)* found no loss of biological value or digestibility of evaporated milk protein during 14 months of storage as measured by the rat nitrogen balance method. Cook et al. *(2)* also reported that storage for 1 year at room temperature did not alter the value of the proteins of evaporated milk as measured by the rat growth method. Since most evaporated milk is consumed within 1 year after processing, it appears that storage losses of protein nutritive value are not serious under the present marketing conditions. Although of little economic importance, the changes in the protein nutritive value of evaporated milk which occur during a prolonged storage of several years

duration are more marked. Hodson *(4)* has shown that after 5 years of storage of evaporated milk there are marked losses of lysine, histidine, and arginine. Rat repletion data on the nutritive value of evaporated milk protein after storage will be presented.

EXPERIMENTAL

Although the production of evaporated milk involves other preliminary heat treatment, the sterilization of the product is the most drastic which it receives. The sterilization temperature is usually 115° to 118°C. for 15 minutes, followed by prompt cooling. While a comparison between the sterilized evaporated milk and the fresh whole milk from which it was made is highly desirable, from a practical viewpoint such a comparison is difficult to make under the conditions of continuous evaporated milk production. By temporarily operating on a batch basis it is possible to prepare evaporated milk from a given lot of fresh milk and preserve samples of each, usually by frozen storage, until the biological investigation has been completed. This introduces the 2 variables, freezing and storage, which do not however, seem to affect the results. Nevertheless, if one studies only the effects of sterilization, the variables can be avoided, if the laboratory is located conveniently to an evaporated milk plant. This investigation was made under the latter conditions. Since evaporated milk is thoroughly mixed and standardized in a storage tank before canning, all cans from a given tank may be considered identical for most purposes. This fact is verified for each tank by check and recheck analyses for butterfat and total solids content. For the investigation samples of non-sterilized evaporated milk in sealed 14½-ounce cans were taken from the processing line entering the continuous sterilizer and samples of sterilized evaporated milk were taken from the line leaving the sterilizer. Samples fed for comparison purposes were always identical except for the effect of sterilization at 115° to 118°C. for 15 minutes. Samples for 1 or 2 days feeding were taken from the commercial processing line and stored under refrigeration until fed. Storage periods of longer than 2 days were not necessary.

The rat repletion method of Wissler, Steffee, Woolridge, Benditt, and Cannon *(10)* was used to compare the proteins of non-sterilized and sterilized evaporated milk. The specific method followed was largely that of Frost and Sandy *(3)* but the non-protein diet was modified by substitution of dextrose for sucrose *(5)*, mineral mixture for U.S.P. No. 1, and Ruffex for Primex. The Ruffex is an aid in the mixing and distribution of the liquids in the diet as well as a source of roughage for the animals.

Both the depletion period during which the non-protein ration was fed and the repletion period during which the ration was supplemented with milk were 12 days in length. The rats were housed in individual wire bottom cages and were supplied the non-protein diet and water ad libitum. There were 3 trials conducted in which the milks were fed at levels of 0.12, 0.18, and 0.24 g. of nitrogen per day. The same rats were used for the 3 trials which were run consecutively without intermission but the groups fed the non-sterilized and sterilized evaporated milk were reversed for the 3 protein levels (Table 1). The animals were paired by weight after each depletion so that the average initial repletion weight of the groups was nearly identical with a maximum average difference of 1.6 g. Adequate pairing and grouping was achieved by shifting only 2 animals between groups.

The supplements were given in liquid form in feeding tubes similar to those customarily used for supplying water to caged rats. No trouble was experienced in getting the depleted rats to consume the supplements. At the lower levels of nitrogen intake they were consumed within a few minutes after feeding.

For the storage trial samples of evaporated milk which had been stored for 16 months and for 5 years at room temperature were available for comparison with recently processed milk from the same plant. The initial processing and the sterilization of the milk at 115° to 118°C. for 15 minutes were alike for the 3 samples. The milk was stored in 14½-ounce tin cans. The storage room temperature was usually in the range 18° to 27°C. but was occasionally higher. These are practical storage temperatures but are slightly higher than found in many warehouses by Monroe, Brighton, and Bendix *(7)*. There were 7 rats used per group. It was the third depletion-repletion cycle for the rats and they were depleted for 16 days in order to compensate for the excessive gain in weight made during 2 earlier depletion-repletion cycles on high quality protein. The

rats were matched by weight in trios and distributed into 3 groups. The initial weight of the 3 groups averaged 228.3, 227.1, and 227.6 g. The animals were then repleted with supplements of the test evaporated milks containing 0.24 mg. of nitrogen per day for 12 days.

TABLE 1

Comparison of protein nutritive value of non-sterilized and sterilized evaporated milk by the rat repletion method

	Grams protein nitrogen fed per rat per day		
	0.12	0.18	0.24
Repletion trial	2	3	1
Number of rats per group	10	10	10
Group number fed non-sterilized milk	IV	V	IV
Group number fed sterilized milk	V	IV	V
Average gain in grams of group fed non-sterilized milk	25.3±1.14	45.9±1.20	70.8±0.95
Average gain in grams of group fed sterilized milk	23.8±0.88	43.4±1.10	71.0±1.03
Difference non-sterilized minus sterilized)	1.5±1.44	2.5±1.63	−0.2±1.40
t	1.04	1.53	0.14
Significance	None	None	None

RESULTS AND DISCUSSION

The results of the comparison of the proteins of non-sterilized and sterilized milk at three levels of nitrogen intake are presented in Table 1. Statistical analysis of the data indicated that the proteins of the non-sterilized milk were not statistically superior in any case and in 1 of the 3 trials the insignificant difference was in favor of the sterilized milk. The rat repletion data therefore support the nitrogen balance data in indicating that no significant loss of protein nutritive value occurs during the sterilization of evaporated milk.

The experiment comparing the proteins of recently processed and stored evaporated milk gave somewhat more significant results (Table 2). The

TABLE 2

Comparison of protein nutritive value of recently processed and stored evaporated milk by the rat repletion method

Rat group	Storage period	Average gain in grams during repletion*	Difference in grams compared to group 1	*t*
1	0	64.9±2.20	.....	.....
2	16 months	58.7±2.12	6.2±3.05	2.03
3	5 years	51.6±2.24	13.3±3.14	4.24

*0.24 g. nitrogen were fed per day.

difference in gain between the groups fed recently processed evaporated milk and those fed evaporated milk stored 16 months is not significant (t is 2.03, but 2.18 is required for significance at the 5% level). The effect of five years of storage is highly significant (t is 4.24, and t equal to 3.06 is required for significance at the 1% level). These data showing no significant loss of protein biological value during the 16 months of storage

are in agreement with the data of Whitnah *(9)* and Cook et al. *(2)*. The results, which suggest loss of protein nutritive value on prolonged storage (5 years), agree with the earlier data of Hodson *(4)*, which show loss of essential amino acids on prolonged storage.

SUMMARY

Investigations carried out by the rat repletion method of protein evaluation indicate that the sterilization of evaporated milk does not significantly reduce the nutritive value of its protein, and that the storage of evaporated milk for 16 months at room temperature, a storage period longer than is usual for this product, did not result in significant loss of protein nutritive value. On the other hand, prolonged storage of a sample of evaporated milk for 5 years at room temperature resulted in a measurable loss of protein nutritive value.

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PHYSICAL CHANGES OF CONNECTIVE TISSUES OF BEEF DURING HEATING[a,b]

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Collagen and elastin are distributed in a fine state of subdivision throughout the muscles of the body. The behavior of these connective tissues is important during the cooking of meat. However, owing to the intimate association of these tissues with the muscle fibers any investigation of their properties is difficult. It is for this reason that the characteristics of collagen and elastin are most easily studied in such tissues as tendons and ligaments.

References in the literature pertaining to collagen are far more numerous than those concerning elastin. Intensive studies have been made of collagen in connection with the leather industry, most of them on skin collagen. Many of these investigations report the effect of acids, alkalies, and salts on the swelling and softening of collagen. References that reported the effect of heating in water on the softening of collagen that might be applied to the cooking of beef were limited. Borasky *(1)* in the introduction to his "Guide to the literature on collagen" states that "when heated gradually in water collagen shrinks within a small but well-defined temperature range."

Tenderness is the physical property of beef in which consumers are most interested. It is well known that the tenderness of beef muscles increases with aging of the carcass or cut. It has been shown that the microscopic appearance of the muscle fibers changes with aging and that these changes accompany the change in tenderness *(2, 4)*. It is not known whether the connective tissues tenderize during the aging of beef. Prudent *(5)* found that the collagen and elastin content of 4 muscles from 2 animals (a yearling steer and an 8-year old dairy cow) did not change by aging portions of each muscle for 1, 2, 4, 10, 20, or 30 days. According to Prudent's results, softening of the connective tissues during aging of the meat would be attributed to physical and not to chemical degradation of these tissues.

The main objective of this study was to determine the effect of heating strips of connective tissue in distilled water at various temperatures and for different periods of time. The physical changes brought about by this treatment were studied in an endeavor to relate the effect of heating connective tissues in water to the cooking of meat. It has been thought that collagenous tissues are softened but that little change occurs in the elastic

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ones during the long, slow moist-heat cookery of less tender cuts of meat. It was found in this study that both elastic and collagenous tissues are softened if heated in water at a sufficiently high temperature.

When beef muscles are cooked they decrease in weight and usually shrink in 2 dimensions (length and width) but expand in the third dimension. The extent of this shrinkage and expansion varies with different muscles and the degree of doneness to which the meat is cooked. The changes in weight, length, width, and thickness of the connective tissues during heating were determined. The extent of softening as obtained by shear force was also determined. All these physical measurements were also obtained on connective tissue from 1 side of 2 animals after aging 10 days and on tissues from the other side of the same animals after aging 35 days.

Total nitrogen, collagen nitrogen, and elastin nitrogen were determined quantitatively on one of each of the tissues, in order to find the relative amounts of collagen and elastin in the various connective tissues used in this investigation. Changes in the histological appearance of the tissues from 2 or more animals at the maximum time and temperature of heating were compared with the unheated tissues.

PROCEDURE

Connective tissues from 9 beef animals were used. The meat from these animals was used for other studies. All animals were procured and killed by the Animal Husbandry Department of the college. The animals identified as 1, 2, 3, 4, and 5 in this study were slaughtered during 1948-1949. Animals 6, 7, 8, and 9 were slaughtered during 1949-1950. For various reasons (tendons not saved from some animals, other tissues used in preliminary tests) data for tendons have been reported only for animals 6, 7, 8, and 9. Only the data for the ligamentum nuchae strips were used from animal 1, and data for the ligamentum nuchae strips from animal 3 were omitted. Data concerning the animals follow:

Animal	Sex	Age in months	Carcass grade	Breed
1	Steer	Unknown	High Commercial	Shorthorn
2	Steer	Unknown	Average Commercial	Shorthorn
3	Steer	Yearling	Average Commercial	Holstein
4	Steer	Unknown	Average Commercial	Angus
5	Cow	72-96	Low Cutter	Holstein
6	Steer	18	High Commercial	Hereford
7	Steer	17	Low Choice	Hereford
8	Steer	5-6	Low Commercial	Hereford
9	Steer	42-48	Average Commercial	Hereford

The connective tissues were removed from the carcasses when the muscles were divided into cuts. This was 24 hours after slaughter for animals 1, 2, 3, 4, and 5; 7 days for animal 8; and 10 days for the remainder of the carcasses.

Connective tissues used. Strips were used from 3 connective tissues. These were 1, a sheet of connective tissue from the flank region; 2, the ligamentum nuchae; and 3, the deep flexor tendon.

The connective tissue from the flank region consisted of a large triangular piece, known as the aponeurotic sheet. The apex of the triangle is the *tuber coxae* (point of hip). In the study 3 portions of this sheet were used. The upper and superficial part of this sheet is the aponeurosis of the external oblique muscle and is hereafter referred to as the external portion of the aponeurotic sheet. The deeper portion of the aponeurotic

sheet is the aponeurosis of the internal oblique muscle and is hereafter called the internal portion of the sheet. The external and internal parts of the tissue fuse to form a sheet which passes superficially to the rectus abdominis muscle. This portion is called the fused part of the aponeurotic sheet.

The ligamentum nuchae is found along the neck and back, is yellow in color and is composed mainly of elastic connective tissue.

The deep flexor tendon is found in the lower part of the leg. It consists almost exclusively of collagenous tissue and is covered by a tough sheath.

Storage and preparation of the connective tissues. After the connective tissues were removed from the carcass, they were labeled (side and animal), then placed in gallon jars of distilled water to prevent drying of the tissues. The tissues (with exception of those from animals 8 and 9) and water were frozen and stored at −17.8°C.(0°F.). The tissues from animals 8 and 9 were aged 10 days by hanging in the carcass in the cooler. The connective tissues from these 2 animals were placed in physiological salt solution with about 0.3% toluene as a preservative. The connective tissues from 1 side of animals 8 and 9 were tested immediately. Those from the other side were stored in the cooler in the solution for 25 days days longer.

To prepare for use the jar of connective tissue was placed in water until thawed. All extraneous fat, loose connective tissue, and muscle fibers were carefully scraped from the main piece.

Each tissue was cut into strips as nearly the same size as possible. The strips had to be small enough to be sheared in an unheated condition under 60 pounds, the capacity of the shearing apparatus. Strips from the aponeurotic sheet section were cut by laying an aluminum plate (containing 9 parallel slots ⅜ inch apart) on the sheet and cutting through the slots with a Christy surgical blade. The slots of the cutting plate were placed as nearly parallel to the direction of the fibers as possible. Thus the width of the strips could be partially controlled but the thickness varied with the variation in thickness of the tissue.

Heating. The strips were heated in a thermostated stainless steel water bath. Once the water bath had reached the desired temperature only negligible variation was noted. A 600-ml. beaker containing distilled water was placed in the water bath. The connective tissue strips were heated in the beaker after the water in the beaker had reached the temperature for the test.

Time of heating. Strips from each of the connective tissues were heated for 1, 2, 4, 16, and 64 minutes at each temperature setting. These times were arbitrarily chosen.

Temperature of heating. Strips from the external portion of the aponeurotic sheet from the ligamentum nuchae were heated at 60°, 65°, 70°, 75°, 80°, 85°, 90°, and 95°C. Strips from the internal and fused portions of the sheet and from the deep flexor tendon were heated at 60°, 80°, and 95°C. There was not enough of these tissues for a greater number of tests.

The temperatures of heating of the tissues used in the aging part of the study follow: 60°, 70°, 80°, 90°, and 95°C. for the external portion of the aponeurotic sheet; the same temperatures for the strips of ligamentum nuchae except 70°C. was omitted; and 80° and 90°C. for the remaining tissues.

Nitrogen determinations. Quantitative determinations of total nitrogen, collagen nitrogen, and elastin nitrogen were made on 1 sample of each tissue to determine, in general, the amounts of collagen and elastin present in the various tissues. The procedure used was a modification of the method of Lowery, Gilligan, and Katersky *(3)*. Owing to time limitations the procedure and technic were not worked out so that the duplicates would check within a given range.

Microscopic sections. Again, owing to time limitations only tissues from animals 2, 5, and some from 4 were used for microscopic sections. The sections from tissues of these animals were prepared from unheated tissues and from tissues heated at 95°C. for 64 minutes. Longitudinal sections were cut 15 to 25 microns thick on a freezing microtome, stained with van Geisen's and Weigert's stains to differentiate collagen and elastin, respectively, then mounted in glycerine jelly.

Shear force. After other measurements were completed, each heated strip was sheared. Six shears were made on each strip with a modified Warner-Bratzler shearing apparatus. A representative unheated strip was sheared as a control for each temperature test.

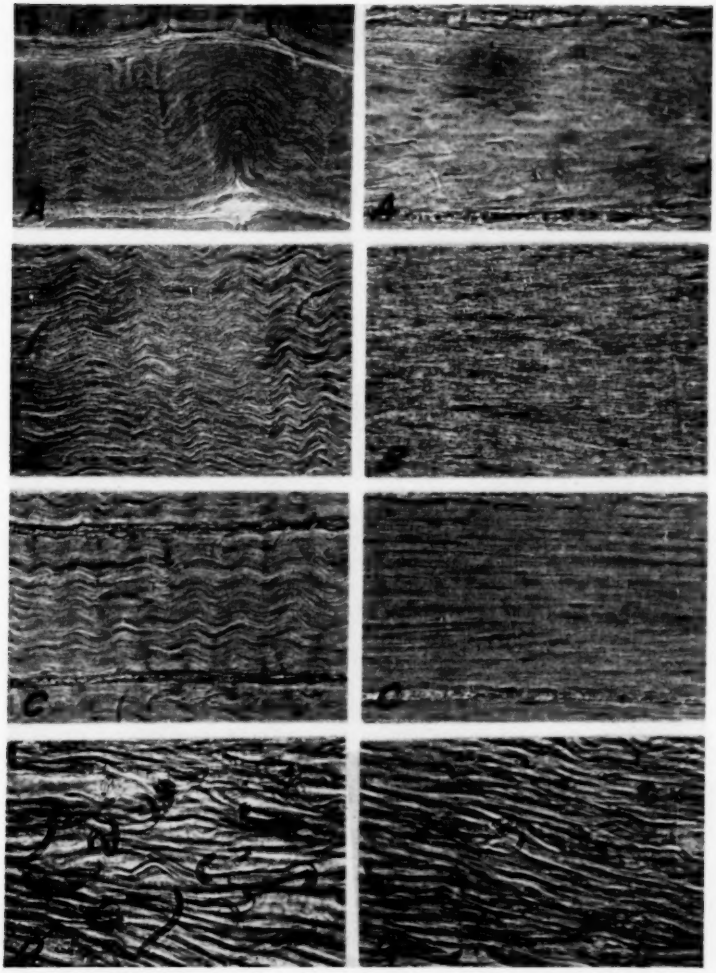

Figure 1. Connective tissues. Left, unheated, right, heated for 64 minutes at 95°C. A, External portion of the aponeurotic sheet (animal 5); B, internal portion of the sheet (animal 5); C, fused portion of the sheet (animal 2); and D, ligamentum nuchae (animal 5). Magnification × 165.

Weight, length, width, and thickness. Each strip was dried thoroughly on a paper towel, then weighed on a balance with a sensitivity of 0.1 g. Length, width, and thickness measurements were taken before and after heating. The thickness was measured by a special device consisting of a rod with a disk on the lower end. The metal rod operated through a fixed ring and was calibrated in mm. The disk was placed on the tissue, which raised the rod a distance above the ring equal to the thickness of the tissue.

RESULTS

Nitrogen analyses. The chemical analysis showed that the deep flexor tendon was mostly collagen with very little elastin, whereas the ligamentum nuchae had the highest percentage of elastin and a smaller amount of collagen. The various portions of the aponeurotic sheet ranged in between 2 extremes. It became evident that the behavior of the connective tissue during heating was related to the proportions of collagen and elastin present (Table 1).

Macroscopic and microscopic appearance. Both the macroscopic and microscopic appearance of the connective tissues varied. The external portion of the sheet was composed of a thin layer of white collagenous fibers on one side, a heavier layer of yellow elastic tissue on the other side. The internal portion of the sheet was white in color and composed largely of collagen; the fused portion consisted of several layers of collagenous and elastic tissue. The internal portion was the thinnest, the fused portion the thickest of the 3 tissues.

The microscopic appearance of the unheated and heated (for 64 minutes at 95°C.) connective tissues are shown in Figure 1. The collagenous fibers of the sheet were in bundles, but the distinctness and width of these bundles varied in different parts of the sheet. Between the bundles were elastin fibers. It was evident that heating produced some change in the microscopic appearance of the collagenous tissue. The heated fibers were straighter, less distinct, and appeared to be fused or merged in some areas.

In the microscopic field a variety of pictures was presented. For example, in some areas of the fused portion of the sheet a layer of green stained elastic fibers was above the differentially red stained collagenous ones. In another area elastic fibers occupied one part of the field and merged into collagenous ones in the other part of the field. In other instances bundles of collagenous fibers were observed to lie at right angles to each other (Figure 2). The collagenous fibers of the deep flexor tendon were coarser than those of the aponeurotic sheet.

The microscopic appearance of the heated elastic fibers was similar to that of the unheated fibers (Figure 1). The unheated elastic fibers from the aged dairy cow were similar to those from the other animals, except the cut ends curled back to a greater extent than in the younger animals. After heating the tendency of the ends to curl back in hooks was not so great.

Physical changes in the connective tissue during heating. Strips composed principally of collagenous tissue when heated at the higher temperatures and longer periods of time became sticky and translucent. The strips shortened in length and became thicker with a tendency to curl (Figure 3). The greatest changes occurred in strips from the deep flexor

TABLE 1

Nitrogen analyses of the connective tissues

Connective tissue	Moisture	Total N (dry wt.)	Collagen N % of total N	Elastin N % of total N
	%	%		
Aponeurotic sheet				
External portion	62.6	15.2	16.4	47.9
	65.0	14.9	19.0	53.1
Internal portion	63.6	14.3	32.7	2.7
	63.3	14.2	25.8	2.9
Fused portion	58.8	12.5	48.0	14.1
	58.9	13.2	41.8	12.3
Ligamentum nuchae	64.6	12.4	16.6	83.7
	64.8	13.4	15.6	80.1
Deep flexor tendon	66.5	16.5	36.7	0.06
	66.5	17.1	34.4	0.08

TABLE 2

Average shear force values of connective tissues. (Each figure is the average of strips from 6 animals, except flexor tendon, which is average of 2 animals at 60° and 4 animals at 80° and 90°C.)

Tissue	Temperature	Minutes of heating					
		0	1	2	4	16	64
	°C.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
Aponeurotic sheet							
External	60	21.1	21.6	19.9	21.0	20.8	19.7
	65	21.2	19.0	18.1	18.1	12.0	12.6
	70	19.4	17.7	17.4	12.8	11.0	9.7
	75	21.2	11.9	11.9	12.6	9.6	10.8
	80	21.9	12.9	11.6	12.0	10.7	8.4
	85	24.6	11.8	10.7	9.6	8.6	9.6
	90	25.9	10.4	10.9	11.4	9.3	8.0
	95	22.6	10.0	9.6	10.4	8.6	7.2
Internal	60	16.4	16.2	18.0	16.7	15.1	13.9
	80	18.3	7.7	9.3	7.3	5.0	4.1
	95	16.0	5.7	5.9	5.6	5.5	2.6
Fused	60	42.2	39.9	41.8	41.5	38.3	38.8
	80	39.1	21.0	22.1	20.2	18.0	15.0
	95	37.3	20.1	17.1	15.7	12.7	7.1
Deep flexor tendon	60	53.2	49.5	50.6	54.0	49.0	51.3
	80	41.6	37.7	33.3	40.0	23.3	21.1
	95	40.0	25.2	31.0	26.6	22.6	11.0
Ligamentum nuchae	60	34.8	33.6	34.1	34.8	35.0	34.1
	65	35.8	33.1	33.0	31.9	31.0	28.5
	70	34.4	34.9	29.9	27.6	28.1	27.8
	75	34.5	27.2	26.7	26.3	27.3	27.4
	80	35.0	28.6	25.0	28.6	24.2	26.0
	85	34.6	27.2	26.7	26.5	25.7	23.9
	90	33.3	27.1	25.5	25.4	25.6	21.1
	95	33.0	25.8	26.4	24.9	24.8	19.0

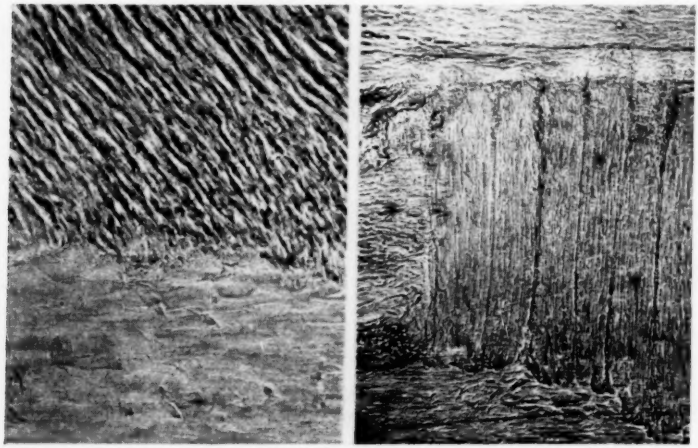

Figure 2. Fused portion of the aponeurotic sheet after heating 64 minutes at 95°C. (animal 5). Left: Elastic fibers at top merging into partially fused collagenous ones. Magnification × 165. Right: Showing varied directions collagenous fibers might take. Magnification × 60.

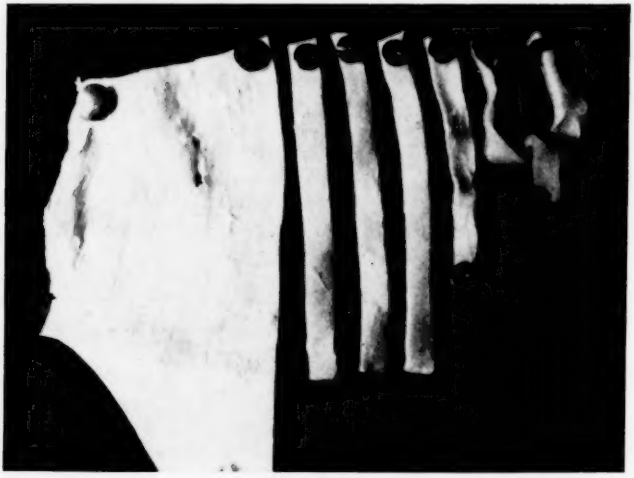

Figure 3. Left: A portion of the external aponeurotic sheet. Middle: Three unheated strips. Right: Three strips heated for 64 minutes at 95°C. in distilled water.

tendon, the least in those from the ligamentum nuchae. The elastic fibers seemed dry and dehydrated after heat treatment.

The differences between the shear force values for the periods of heating, the temperatures of heating, animal variations, and the interaction of heating periods and heating temperatures were all significant at the 1% probability level for the data obtained on the external portion of the aponeurotic sheet. Data for the other tissues followed the same general pattern as those from the external portion of the sheet. However, when the data for strips heated at each temperature were considered individually, it was observed that a linear relationship did not always exist between shear force values and time of heating (Table 2). In general, the connective tissues containing more collagen softened to a greater extent than those containing more elastin. Although the general pattern of tenderizing of the ligamentum strips was similar to that of the external portion of the sheet, this tissue was always characterized by less softening at any given temperature and time period than for the tissues containing more collagen.

The extent of softening varied considerably with the temperature of heating. In general, there was little or no softening at 60°C., even after 64 minutes of heating. The critical temperature for softening of the connective tissues seems to be around 65°C. This statement is based on data for the strips from the external sheet and the ligamentum nuchae, since these were the only tissues heated at 65°C. At 65°C. the softening of the strips was slight for the shorter periods of heating but much greater after heating 16 and 64 minutes (Figure 4).

The extensive softening, shown by the small number of pounds for shearing all strips heated only 1 minute at 80°C. and higher temperatures, seems remarkable (Figure 4). Ramsbottom, Strandine, and Koonz *(6)* compared the tenderness of 25 representative beef muscles before and after cooking. The beef muscles were cooked in lard held at 121.1°C.(250°F.) until the interior temperature of the muscle reached 76.7°C.(170°F.). The muscles cooked in a shorter time by this method than when cooked in an oven at 162.8°C.(325°F.) and were more tender by the latter method of cooking. Most of the 25 muscles were less tender after cooking than when raw. This suggested that the denaturation and coagulation of the fiber proteins by cooking contributed to the toughness of the meat.

Aging the connective tissues from one side of the carcass for 10 days and those from the other side for 35 days brought about differences in shear force. The tissues aged the longer time required slightly less pounds for shearing than those aged 10 days. The increase in tenderness with aging 25 days longer was not great, but, in general, the results were consistent (Table 3).

Muscles from old animals are expected to be less tender than those from young ones. Hence, the average shear force values of the unheated connective tissues from 6 of the animals are of interest (Table 4). It should be recalled that connective tissues from animals 2, 3, 4, and 5 were removed from the carcass after 24 hours of chilling, the tissues from animals 6 and 7 after 10 days in the cooler. The tissues from the 8-year old cow usually required considerable shear force but in no instance were they

TABLE 3

Average shear force values for tissues (averages for all temperatures at which tissue was heated) for animals 8 and 9

Tissue	Minutes of heating					
	0	1	2	4	16	64
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
External sheet						
Aged 10 days	18.1	14.5	13.6	12.4	10.1	9.8
Aged 35 days	17.4	11.5	12.2	12.2	10.3	8.8
Internal sheet						
Aged 10 days	22.2	6.2	5.2	5.7	4.4	2.8
Aged 35 days	16.9	4.6	2.7	2.2	1.8	0.5
Fused sheet						
Aged 10 days	44.6	22.5	21.2	19.5	16.2	10.1
Aged 35 days	38.5	18.9	16.2	16.2	15.9	9.5
Ligamentum nuchae						
Aged 10 days	35.0	29.2	29.0	30.0	29.1	26.6
Aged 35 days	27.8	25.8	25.6	24.2	24.1	23.2
Deep flexor tendon						
Aged 10 days	44.4	24.5	28.8	35.5	21.2	16.5
Aged 35 days	48.0	22.5	23.6	29.0	18.8	16.2

TABLE 4

Average shear force values of connective tissues from six animals

Tissue	Animal No.[c]					
	2	3	4	5 (cow)	6	7
	lbs.	*lbs.*	*lbs.*	*lbs.*	*lbs.*	*lbs.*
External sheet	36.6	18.8	17.3	21.9	20.6	18.1
Internal sheet	.....	16.7	18.3	17.5	14.9	16.4
Fused sheet	33.5	32.9	37.2	44.7	46.9	35.4
Ligamentum nuchae	46.1	.....	31.8	38.2	28.2	31.4
Flexor tendon	.....	.....	.....	.....	42.1	48.5

[c]Age of animals 2 and 4 unknown; of animal 3, a yearling; of animal 5, 72-96 months; of animal 6, 18 months; and of animal 7, 17 months.

TABLE 5

Average shear force values of unheated tissues from the calf and the 3.5-4 year old steer. The figures for thickness are averages of the thinnest and thickest portions of the strips

Tissue	Calf, 5-6 months old		3.5-4-year-old steer	
	Thickness of tissue	Shear force	Thickness of tissue	Shear force
	mm.	*lbs.*	*mm.*	*lbs.*
External sheet	1.2-1.6	8.8	1.4-1.8	16.2
Internal sheet	.....	3.9	.....	8.6
Fused sheet	1.1-1.6	14.7	2.0-2.5	26.8
Ligamentum nuchae	3.7-4.4	24.6	4.4-5.5	30.4
Flexor tendon	1.8-2.6	25.6	2.0-3.1	29.2

the highest. If one assumes that age of animal and aging were the only factors which contributed to the differences, the data in Table 5 indicate that animal age as well as aging of the tissue had an effect upon the shear force values in this instance. Variability in thickness of the tissues may have accounted for part of the difference in shear force required for the

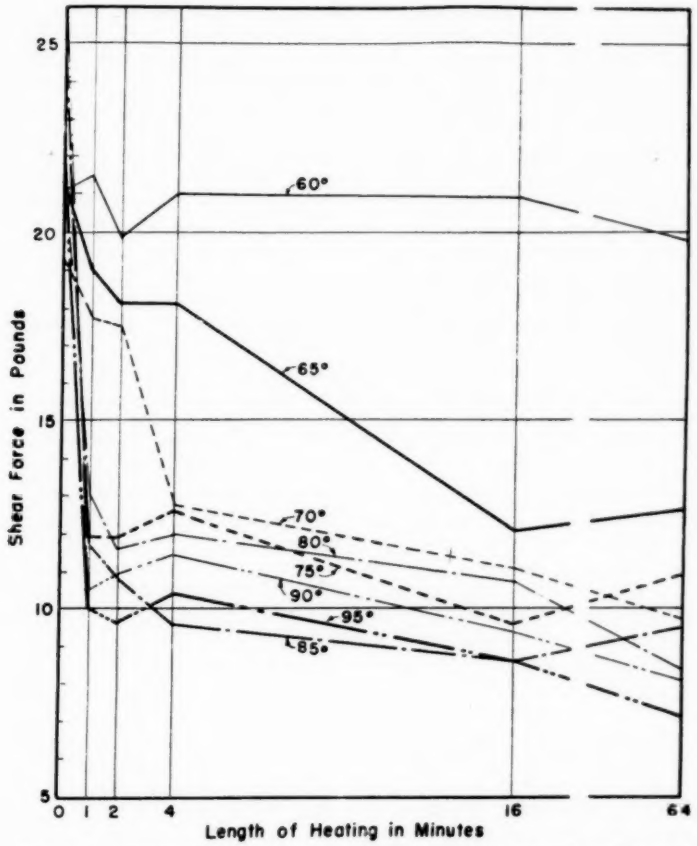

Figure 4. Average shear force values of strips from the external portion of the aponeurotic sheet.

tissues from animals 8 and 9. But, after allowances for differences in thickness of the tissues and for aging of the tissues, it seems that age of the animal may have had considerable effect upon the shear force values, particularly for tissues of the aponeurotic sheet. Since the tissues for animals 8 and 9, Table 5, were aged for similar periods of time, the data

obtained with them may represent the effect of animal age better than the data in Table 4.

Although all of the factors investigated were, in general, dependent on the time and temperature of heating and on the relative amounts of collagen and elastin which were present in the tissues, they also varied with the animal from which the tissue was obtained. Throughout the investigation sampling variation was large. Measurements of width and thickness were difficult to make on the heated strips as the edges of many of the tissues curled inwards. In addition, there was considerable variation in the amount and character of the collagenous fibers in each tissue.

Nearly all the strips from each connective tissue lost weight when heated in water. This loss in weight increased with longer periods of heating for the ligamentum nuchae. The strips containing a larger proportion of collagen than elastin lost most of their weight within the first 2 minutes of heating, little change taking place during the rest of the heating period. However, tissues with the largest proportion of collagen tended to absorb water and regain some weight with the longer periods of heating. The deep flexor tendon strips were heavier after 64 minutes of heating at 60° and 80°C. than at the start of the tests. No comment is needed for the data on the decrease in weight of the external portion of the aponeurotic sheet, given in Table 6.

TABLE 6

Average decrease in weight of tissues from the external portion of the aponeurotic sheet and the deep flexor tendon when heated in water

Tissue	Temperature	Minutes of heating				
		1	2	4	16	64
	°C.	%	%	%	%	%
External portion	60	12.1	12.1	10.4	14.3	9.1
	65	12.2	14.8	16.8	16.7	14.3
	70	2.6	7.2	10.8	12.6	16.3
	75	8.1	13.6	15.7	12.2	14.8
	80	18.4	16.6	12.4	7.1	13.4
	85	19.9	14.1	13.9	11.2	13.8
	90	13.2	18.4	12.9	13.1	16.1
	95	17.6	17.7	19.9	13.2	16.0
Flexor tendon	60	7.7	4.2	7.1	3.6	+5.0[d]
	80	11.1	7.0	4.4	6.0	+2.5
	95	4.1	21.4	21.8	9.2	10.1

[d] A plus sign indicates a gain in weight.

Most of the tissues shortened in length, decreased in width, and increased in thickness when heated in water. Strips from the deep flexor tendon increased in both width and thickness. Shortening in length was slight at 60°C. and also at 65°C. for the short heating periods. The shrinkage was considerable for the two longer periods at 65°C. (Table 6). The greatest decrease in length for the ligamentum nuchae for any time or temperature (16 minutes at 95°C.) was 11.5%, for the tendon 64.6% (16 minutes at 95°C.).

Figures for changes in width and thickness for any of the tissues are omitted. They followed the same general pattern of other changes but the

variation was greater. The greatest decrease in width for any time and temperature of heating was 6, 31, and 13% for the external portion of the sheet, the fused portion, and the ligamentum nuchae, respectively. The greatest gain in width for the flexor tendon was 72% for 64 minutes of heating at 80°C.

The greatest increase in thickness (all at 80°C. for either 16 or 64 minutes of heating) for the connective tissues in the same order as in the preceding paragraph was 60, 87, 8, and 140%.

The connective tissues aged 35 days behaved similarly to other tissues in respect to changes in weight, length, width, and thickness.

TABLE 7

Average decrease in length of strips from the external portion of the aponeurotic sheet when heated in water

Temperature	Minutes of heating				
	1	2	4	16	64
°C.	%	%	%	%	%
60	2.2	2.7	2.8	3.9	4.4
65	2.7	3.7	6.4	21.0	19.9
70	18.2	21.3	26.6	22.0	29.6
75	22.7	28.3	29.4	31.3	31.9
80	31.7	30.1	32.0	24.4	30.8
85	33.2	30.2	28.4	29.6	27.5
90	31.4	30.5	32.5	27.3	22.8
95	30.5	32.2	34.0	32.0	29.8

Application of findings to cooking of meat. Little softening or other physical change of connective tissues occurred at 60°C. Likewise at 65°C. little change occurred with the short heating periods but heating for 16 and 64 minutes brought greater changes. If the connective tissues in muscle are similar to the tissues in this study, these results may be interpreted to mean that cooking of steaks and roasts to rare and medium done states (55° to 65°C.) brings about little if any change in the connective tissues of the muscles. Both collagenous and elastic tissues were softened by heating in water at the higher temperatures. However, since the elastin content of most muscles is small compared to the collagen content, it seems that the tenderness of cooked meat is affected to a greater extent by collagen than by elastin.

Even though collagen is softened by heating it must be emphasized that heating also coagulates the muscle proteins and hardens the muscle fibers. These factors are all intimately associated with the tenderness of cooked meats. The speed with which the connective tissues softened in this study suggests that less tender braised cuts of meat are cooked a long time in moist heat to interior temperatures of 80° to 95°C. to soften the coagulated fibers as well as the connective tissues. The shortening in length, decrease in width, and increase in thickness (at higher temperatures this may be followed by a decrease of thickness) of meat during cooking may, at least in part, be affected by the collagen content of the muscle.

SUMMARY

Representative connective tissues from 9 animals were used. Collagen and nitrogen determinations were made on one of each of the tissues to determine the relative proportion of collagen and elastin in each tissue. The tissues were the ligamentum nuchae (principally elastin), the deep flexor tendon (principally collagen), and the aponeurotic sheet (consisting of the external, internal, and fused portions with varying amounts of collagen and elastin).

The tissues were cut into strips and the effect of heating in distilled water for 1, 2, 4, 16, and 64 minutes at varying temperatures from 60° to 95°C. upon the physical changes of shear force, weight, length, width, and thickness were determined.

Heating for 64 minutes at 95°C. brought about some change in the microscopic appearance of collagenous but apparently little or none in the elastic tissues. The heated collagenous fibers appeared merged or fused in some areas and were also straighter and less distinct than the unheated ones.

Connective tissues softened (as shown by pounds for shearing the strips), lost weight, shortened in length, decreased in width, and increased in thickness during heating. These changes occurred together, and, in general, the degree of changes increased as the time and temperature of heating was elevated, and as the collagen content of the tissues increased. Little or no change occurred at 60°C. even with 64 minutes of heating. At 65°C. little change occurred with the short periods of heating. With the higher temperatures the physical changes occurred rapidly. Variation also occurred owing to inherent differences in connective tissues from different animals. Aging tissues from 1 side of 2 animals for 35 days as compared with 10 days of aging for tissues from the other side increased the tenderness of the connective tissues only slightly.

Acknowledgment

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Coliform Bacteria

BACTO-VIOLET RED BILE AGAR

is recommended in "Standard Methods for the Examination of Dairy Products" for the direct plate count of the coliform bacteria. This medium is especially prepared for direct enumeration of coliform bacteria in water, milk and other dairy or food products. Upon plates of medium prepared from this product subsurface colonies of the coliform types are generally surrounded by a reddish zone of precipitated bile. Due to the inhibitory action of the medium toward other types accurate counts are obtained after incubation for only 18 hours.

BACTO-BRILLIANT GREEN BILE 2%

is recommended for the detection of coliform bacteria. This medium conforms in every way to the brilliant green lactose peptone bile described in "Standard Methods for the Examination of Dairy Products" and in "Standard Methods of Water Analysis" of the American Public Health Association. Results obtained by the direct inoculation of water, milk and dairy products or other food materials into fermentation tubes of this medium are reliable and accurate.

BACTO-FORMATE RICINOLEATE BROTH

is also employed for the detection of coliform bacteria. The medium is used in fermentation tubes which are inoculated directly with the sample or dilution. Bacto-Formate Ricinoleate Broth conforms to the "Standard Methods" formula.

Specify "DIFCO"

THE TRADE NAME OF THE PIONEERS
In the Research and Development of Bacto-Peptone and Dehydrated Culture Media

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